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DRYING OF ROUGH RICE WITH HEATED AIR IN FLAT BED DRYERS [1]

ALEJANDRO B. CATAMBAY, DANTE B. DE PADUA AND JOSE R. ARBOLEDA

The drying air temperature affects the quantity and quality of milled rice recovery. A significant decrease of 3.3 per cent in total milling recovery was found when the drying air temperature was increased from 120°F to 140°F.

In a grain bed 17 inches thick, there was no significant variation in the total milling recovery between samples taken from the top, center, and bottom, up to a drying air temperature of 140°F. At 160°F a significant variation was found with a lower total milling recovery at the bottom. In the case of head grain recovery however, a significant variation, as shown by a lower recovery at the bottom, was already found at 120°F.

The decrease in total milling recovery at 160°F is explained by the significant increase in Brewer's rice recovery. A significant variation was also found in Brewer's rice recovery among samples taken at the different depths of the grain bed at drying air temperatures of 140°F, with more Brewer's rice recovered from samples taken at the bottom.

An increase in drying air temperature substantially increases the rate of removal of moisture, but with a corresponding increase in fuel consumption. There is, however, a decrease in the percentage of air utilization as the temperature is increased.

The use of grain dryers employing heated air affords a high degree of control on the drying conditions. It also makes the conditioning of the grain completely independent of prevailing weather conditions. Sun drying of harvested grain, while effective, is applicable only on limited quantities of grain. Irrigation has made possible the growing of a second crop harvested during the rainy months of the year, thus making the use of grain dryers a requisite for the safe storage of the grain.

This study is based on the premise that the external variables during the drying process affect the final quantity and quality of the milled rice recovered. The external variable conditions that may be specified or controlled in the design or operation of a drying sys-

[1] Central Experiment Station Contribution No. 2042. Research Project No. 18 of the Rice and Corn Production Program.

tem, and which affect the drying process are the drying air temperature, humidity, drying air flow rate, the time of exposure of the grain to the hot air drying stream, and the depth of the grain bed.

Fluid flow within the grain during the drying process has its particular value in improving performance and increasing the capacity of the drier. However, the results thus far gathered consider the effects of the external variables with more direct application in the design and operation of the equipment.

This study was made primarily to determine the effect of the drying air temperature on the final quantity and quality of milled rice recovered under conditions normally existing in a commercial flat bed dryer.

A GENERAL DISCUSSION OF GRAIN DRYERS

Types of grain dryers. The conditioning of harvested grain for proper storage and milling is conveniently accomplished in batch or continuous flow dryers with heated or unheated air forced through the grain mass. The batch dryers, where the grain is contained in the drying bin until it is dried, may be of the deep bed or shallow bed type. Drying is a single continuous process, kept on until the grain moisture has reached a safe storage level, usually 14 per cent.[2] Continuous flow dryers are usually of the columnar type where drying is an intermittent process, allowing the grain to temper in between exposures to the hot air stream, and in effect decreasing the total drying time and therefore increasing the drying plant's capacity. This is more popular, but because of the mechanical facilities required for loading and unloading the grain, the initial cost of this equipment is considerably higher than others.

Batch drying in flat beds, preferably where the grain mass is less than 24 inches deep, has greater possibilities for Philippine farms. The drying bin may be constructed by local carpenters from locally available materials. The blower and heating units may also be made in local machine shops. The flat bed arranged horizontally or slightly inclined to facilitate unloading may be manually loaded and unloaded. The grain bed is placed above an airtight enclosure serving as plenum to diffuse the air uniformly through the grain mass.

The drying theory and its fundamental concepts. The drying process involves, like problems in refrigeration or air-conditioning,

[2] All moisture contents in this paper are expressed on a wet basis.

an understanding of the properties of the air.[3] A mixture of water vapor or technically, steam in a superheated state with dry air in the atmosphere is known as humidity. Relative humidity is the ratio of the partial pressure of the water vapor in the air to the pressure which saturated water vapor exerts at the temperature of the air. Absolute humidity is the weight of water vapor expressed in pounds or grains of water vapor associated with each pound of dry air. The maximum amount of water vapor that a given space can hold depends on the temperature. The degree of saturation or the relative humidity decreases as the air is heated. Prediction of conditions of the various properties of the air and steam mixtures in the analysis of a process is conveniently done with the aid of steam tables and psychrometric charts. Psychrometric charts present in graphic form the properties of the air under different temperature and humidity conditions.

Crop grains, being hygroscopic in nature, will gain or lose moisture under a given set of air temperature and humidity conditions. Thermodynamically, a product is in equilibrium with its environment when the moisture retained within the grain, termed equilibrium moisture content, is in a state of equilibrium with the water vapor in the surrounding air. The free energy exchange is zero. The differential in vapor pressure between the moisture in the grain and the water vapor in the atmosphere is the driving force in the transfer of moisture. If the vapor pressure within the grain is higher than that of the surrounding atmosphere, the moisture will move from the grain to the atmosphere.

In Philippine climate where the relative humidity is high most of the year, the air must be heated before it is forced through the grain mass. Heating of the air lowers its relative humidity and increases its moisture carrying capacity. The reasons for utilizing the heated air during the drying process are: first, it increases the temperature of the grain to the wet bulb temperature of the drying air, the condition at which the moisture is vaporized, and second, it serves as the vehicle for carrying away the vaporized moisture. Therefore, as the heated air moves through the grain mass the resultant drying process is that, heat is transferred to the grain and the moisture in the grain is absorbed by the drying air, increasing its absolute humidity. The process is known as a simultaneous

[3] S. M. Henderson and R. L. Perry, "Agricultural Process Engineering," John Wiley and Sons, Inc., New York, 1955, pp. 273-300.

heat and mass transfer, or an adiabatic process in which the sensible heat decrease in the air is balanced by the latent heat of the water which evaporates into it, and the original wet bulb temperature of the air remains unchanged.

Another important factor that influences air utilization in the drying process is the drying response of the grain at different moisture levels. In this respect, the drying process is divided into two periods—the constant rate period, and the falling rate period.[4] During the constant rate period, the grain loses moisture in a manner typified by evaporation from a free water surface on the surface of the grain. This is essentially independent of the grain. The rate of drying at this stage depends chiefly on diffusion of the heat and vapor through the air film at the surface of the grain into the main body of the drying air stream. Air velocity, or here called air flow rate, is the principal factor in varying the thickness of this film. In this study, the air flow rate is controlled at a constant value.

The falling rate period is entered after the constant rate period, during which the drying rate depends upon the rate of diffusion of the moisture from within the grain to the surface where it can be vaporized. The moisture content at the end of the constant rate period is called the critical moisture content. In a continuous drying process, drying occurs mostly under the falling rate period, and the time of response has been compared to the time of radioactive decay or disintegration.[5] Drying rate is non-linear; it decreases as the moisture content of the grain approaches equilibrium conditions. When only one-half of the original material still remains, the time in hours from the beginning is known as the time of one-half life or response. It would take the same time to disintegrate one-half of what was left and so on. The same terminology is applied to heat transfer and moisture movement under the falling rate period. The difference between the critical and equilibrium moisture contents represents the original material available for change. Thus the time for one-half response would be the number of hours necessary to obtain a moisture content halfway down to equilibrium conditions. The significance of this is that as the drying progresses, the drying capacity of the air is not fully

[4] John H. Perry, "Chemical Engineers' Handbook," McGraw-Hill Book Company, Inc., New York, 1950, pp. 799-884.

[5] Carl W. Hall, "Drying Farm Crops," Edwards Brothers Inc., New York, 1957, pp. 17-44.

utilized. The percentage of air utilization is important in the design of drying systems.

MATERIALS AND METHODS

The experimental dryer. Research work on a commercial scale drying operation would be ideal. This, however, requires huge quantities of grain. Work was done with an experimental dryer designed to simulate the drying conditions on a commercial dryer. Four circular bins each with a floor area of four square feet (total, 16 sq. ft.) and a capacity to hold a maximum depth of two feet of grain were used. Measurements were made on four bins, each representing a trial. The bins were seated on a plywood enclosure serving as the plenum to diffuse the drying air uniformly through them. The blower was connected to the plenum chamber with a duct. The fan and heating system used was a HABCO unit, an indirect-fired type provided with a heat exchanger and a 28-inch, 8-blade propeller type fan driven by a 5 HP, 3 phase, 220 VAC 1750 rpm electric motor. It was provided with an oil burner of the atomizing jet type which had a fuel pump and interchangeable nozzles. The HABCO unit was also provided integrally with temperature and other safety automatic controls.

Experimental design and procedure. Drying air temperature treatments measured at the plenum chamber of 100°F, 120°F, 140°F, 160°F, and 180°F were used. A suitable fuel burner nozzle for 180°F was not available so that the very high temperature of 180°F could not be constantly maintained. The data obtained therefrom were discarded. Air-drying, that is, spreading the grain on the floor inside the building, comprised one of the treatments. Sun-drying intended as another treatment could not be performed because of unfavorable weather conditions at the time of the study.

Palay of the Azucena variety harvested at the Experiment Station farm at an average moisture content of 23 per cent w.b. (wet basis) was cleaned and used. Two cavans of 44 kilograms each were loaded on each of the four bins for every temperature treatment. This quantity gave a depth of 17 inches of grain.

Air flow rate was maintained constant for all temperature treatments. This was checked and controlled indirectly by measuring the static pressure at the plenum chamber with an inclined gauge manometer. Drying of each temperature treatment was done con-

tinuously until the grain at the top of the bin was measured and found to have an average moisture content of 14 per cent w.b.

During each drying operation the following instrumentations were performed and recorded:

(1) Using an All-American Crop Moisture Meter, the initial moisture content of the grain was measured. The meter is a direct weighing type with a high wattage heater lamp to vaporize the moisture from the ground sample.

(2) The static pressure at the plenum was measured and maintained at a constant value by adjusting a damper on the intake duct of the plenum. From these data, and from data published in the Agricultural Engineer's Year Book (1959),[6] the total air-flow rate was estimated.

(3) The relative humidity and the ambient air temperature in the drying area were measured morning, noon, and afternoon during each day of operation. The mean value was used in the thermodynamic analysis of the drying process.

(4) The initial and final weights of the fuel drum were measured before and after each drying operation.

(5) A spot check on the moisture content of the grain was done with a CERA electronic moisture meter. The meter was of the variable capacitance type.

(6) During the drying process, the temperature at the plenum, at the exhaust, and at every six inches of grain bed was measured. This indicated to the drying operator the degree of saturation of the drying air and indirectly, the drying progress.

(7) After each drying operation and after allowing the grain to cool overnight, final moisture content at the top, center, and bottom of the bins were measured with the CERA electronic moisture meter.

(8) Samples for each treatment were taken from the top, center, and bottom of the bin and tested in the Standard McGill Laboratory Sheller and Polisher, for total milling recovery, head grain recovery, and Brewer's rice recovery.

(9) The total actual drying time was also recorded.

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[6] *Agricultural Engineers Yearbook*, American Society of Agricultural Engineers, St. Joseph, Michigan, 1959. p. 115.

RESULTS AND DISCUSSION

The drying rate as influenced by the drying air temperature has a significant effect on the total milled rice recovery and the head grain recovery.

Table 1 shows that there are no significant differences in milling recovery between air dried treatment and temperature treatments up to 140°F. However, the decrease in milling recovery at 160°F is significantly different.

TABLE 1.—*Percentage of total milled rice recovery (Average of four trials)*

TEMPERATURE	DEPTH			
	Bottom	Center	Top	Average
Air dried	—	—	—	64.8
100°F	67.0	66.9	63.8	65.9
120°F	64.9	64.8	63.9	64.5
140°F	65.0	64.8	64.9	64.9
160°F	59.3	66.2	61.3	61.6
Average	64.1	65.7	63.5	

H.S.D. Temperature .. 2.16 at 1% probability level; 1.77 at 5%
Depth ..... 1.63 at 1% probability level; 1.28 at 5%

Table 2 shows that there are no significant differences in head grain recovery between air dried treatment and temperature treatment up to 120°F. At 140°F and higher, however, the decrease in head grain recovery is significantly different.

TABLE 2.—*Percentage of head grain recovery (Average of four trials)*

TEMPERATURE	DEPTH			
	Bottom	Center	Top	Average
Air dried	—	—	—	46.0
100°F	50.9	50.6	48.0	49.8
120°F	35.9	46.7	40.7	41.1
140°F	15.0	27.6	31.7	24.8
160°F	9.2	14.2	39.0	20.8
Average	27.8	34.8	39.9	

H.S.D. Temperature .. 10.11 at 1% probability level; 8.28 at 5%
Depth ..... 7.86 at 1% probability level; 6.19 at 5%

These results indicate that under this particular system and condition of drying, the higher drying air temperature limit for the

production of quality grade milled rice, would be somewhere between 120°F and 140°F.

But the decrease in milling recovery at 160°F is not a complete loss. Table 3 shows that there is a significant increase in brewer's rice recovery. Therefore, the loss in milling recovery may be attributed to the checking of the grain during the drying process. It is recovered in the milling process as Brewer's rice.

TABLE 3.—*Percentage of brewer's rice recovery (Average of four trials)*

TEMPERATURE	DEPTH			
	Bottom	Center	Top	Average
Air dried	—	—	—	4.3
100°F	4.6	4.5	4.7	4.6
120°F	6.0	5.3	5.0	5.4
140°F	6.7	4.5	3.8	5.0
160°F	9.8	6.6	5.7	7.4
Average	6.8	5.2	4.8	

H.S.D. Temperature .. 1.90 at 1% probability level; 2.32 at 5%
Depth ..... 1.79 at 1% probability level; 1.41 at 5%

There is a significant variation in the total milling recovery and head grain recovery at different depths in the 17-inch grain bed. Table 1 shows that there is a lower milling recovery from the samples taken from the bottom than from the center and the top at 160°F. Table 2 shows that there is a lower head grain recovery from the samples taken from the bottom than from the center and top at 120°F, 140°F, and 160°F.

These results may be explained by the fact that the grain at the bottom is exposed longer to the hot air stream with lower relative humidity and consequently, it is dried down to lower moisture contents than the grains above it. Table 4 shows that at drying temperatures of 120°F, there already exists a moisture content differential of from 9.02 per cent at the bottom to 13 per cent at the top, and a bigger diferential at the higher temperature treatments. This indicates that under this particular air-flow rate and drying conditions, a thinner drying bed is desirable; it may be even half of the 17 inches.

An analysis of the different factors affected by the variation in drying air temperature (table 4) indicates that an increase in drying air temperature substantially increases the drying rate. The

increase in drying rate shown as kilograms of moisture removed from the grain per hour, means shorter drying time and higher drying capacity for the drying system. However, an increase in drying air temperature increases fuel consumption, expressed here as kilograms of fuel burned per hour. Also, there is a decrease in air utilization.

TABLE 4.—*Drying history*

	TEMPERATURE [a]				
	Air dried	100°F	120°F	140°F	160°F
Ave. initial moisture content, %	22.8	20.8	21.3	22.0	21.7
Final moisture content, %					
Bottom	—	*	9.02	6.27	6.20
Center	—	*	13.00	13.85	14.42
Top	—	*	13.00	13.55	14.42
Ave.	—	*	11.23	10.13	10.27
Moisture removed, kg.	10.85	*	9.98	13.11	11.24
Actual drying time, hours	(10 days)	9.08	4.33	4.08	3.25
Drying rate, kg. moisture/hour	0.045	*	2.30	3.21	3.45
Actual fuel consumption. kg./hr.	—	1.98	5.59	9.24	11.51
Air utilization %, Theoretical drying time/Actual drying time		—	40.5	39.3	33

[a] Static Pressure = 0.66 inches of water (constant at all drying temperatures.
* No data.

Drying air utilization is computed as the ratio in per cent of the theoretical drying time to the actual drying time. This indirectly expresses the amount of utilization of the moisture absorbing capacity of the drying air. Air at ambient conditions when heated to the drying temperature drops in relative humidity and therefore, increases its moisture absorbing capacity but maintains its absolute humidity. As the drying air is forced through the grain mass, it absorbs moisture and under favorable conditions, is saturated. Thus, for a given weight of moisture to be vaporized and carried away, the theoretical amount of drying air required, whose absorbing capacity is fully utilized, can be calculated. The fact that in the actual process more air is used and that the exhaust air is not at saturation temperature during the latter stages indicates that the absorbing capacity of the air is not fully utilized. The theoretical volume of air required in cubic feet divided by the actual air flow rate in cubic feet per minute would give the theoretical drying time if the air were fully utilized. Thus, the ratio of the theoretical drying to the actual time would be an expression of the air utilization.

$$\% \text{ A.U.} = \frac{V_t \ 100}{V_a} = \frac{V_t \ 100}{Q_a \times T_a}$$

$$\frac{V_t}{Q_a} = T_t$$

$$\% \text{ A.U.} = \frac{T_t \times 100}{T_a}$$

% A.U. = per cent air utilization
V_t = theoretical volume of air required, ft.^{3}
V_a = actual volume of air used, ft.^{3}
Q_a = Air flow rate, ft.^{3}/min.
T_a = Actual drying time
T_t = Theoretical drying time for full air utilization.

RECOMMENDATIONS

Pending further research developments, it is recommended that the drying air temperature for continuous drying in flat bed dryers should be below 140°F in order that the quality of the grain may not be affected during the process and, that the depth of the grain mass should be decreased from the 17 inches used in this experiment. A depth of eight inches is tentatively recommended.

APPENDIX A—STATISTICAL ANALYSIS

TABLE A.—*Percentage of recovery*

TRIAL	Air dried	TEMPERATURE											
		100°F			120°F			140°F			160°F		
		T	C	B	T	C	B	T	C	B	T	C	B
I	66.0	64.0	67.5	66.0	62.5	64.0	64.0	65.5	62.0	66.0	60.0	65.8	59.0
II	64.0	64.0	67.0	67.5	63.0	65.0	64.5	64.5	66.0	66.0	60.0	60.0	60.0
III	65.0	63.0	66.0	66.5	65.0	64.0	66.5	63.0	65.5	66.0	62.0	67.0	60.0
IV	64.0	64.0	67.0	68.0	65.0	66.0	64.5	66.5	65.5	62.0	63.0	64.0	58.0
Total	259.0	255.0	267.5	268.0	255.5	259.0	259.5	259.5	259.0	260.0	245.0	256.0	237.0
Ave.	64.8	63.8	66.9	67.0	63.9	64.8	64.9	64.9	64.8	65.0	61.0	64.2	59.3

Analysis of variance

S.V.	D.F.	M.S.	COMP. F	PROB.
Temperature	4	31.24	14.20	<.01
Airdried vs. bindried	1	1.10	.50	
Among bindried	3	41.29	18.76	<.01
Depth	2	12.00	5.45	<.01
Depth × bindried T	6	9.02	4.10	<.01
Error	39	2.20		
Total	51			

	H.S.D.	
	.05	.01
Depth	1.28	1.63
Temperature	1.77	2.16

TABLE B.—*Percentage head grain recovery*

TRIAL	Air dried	TEMPERATURE											
		100°F			120°F			140°F			160°F		
		T	C	B	T	C	B	T	C	B	T	C	B
I	47.4	48.9	49.3	51.4	41.5	44.9	41.2	31.8	28.1	8.5	38.3	19.9	6.7
II	45.0	47.6	51.4	52.9	39.5	45.8	32.9	39.6	38.8	22.0	33.6	4.2	22.7
III	47.0	48.3	52.2	48.6	44.0	47.6	37.6	14.8	27.1	15.0	42.3	24.2	3.5
IV	44.3	46.9	49.7	50.5	37.9	48.3	31.8	40.5	16.3	14.4	41.6	8.3	4.0
Total	184.1	191.7	202.5	203.4	162.9	186.6	143.5	126.7	110.3	59.9	155.8	56.6	36.9
Ave.	46.0	48.0	50.6	50.9	40.7	46.7	35.9	31.7	27.6	15.0	39.0	14.2	9.2

Analysis of variance

S.V.	D.F.	M.S.	COMP. F	PROB.
Temperature	4	1,812.44	35.94	<.01
Airdried vs. bindried	1	525.06	10.41	<.01
Among bindried	3	2,241.5	44.44	<.01
Depth	2	589.50	11.69	<.01
Depth × bindried T	6	197.75	3.92	<.01
Error	39	50.43		
Total	59			

	H.S.D.	
	.05	.01
Depth	6.19	7.86
Temperature	8.28	10.11

TABLE C.—*Percentage of brewer's rice recovered*

TRIAL	Air dried	TEMPERATURE											
		100°F			120°F			140°F			160°F		
		T	C	B	T	C	B	T	C	B	T	C	B
I	4.2	5.0	5.0	4.9	4.3	5.2	6.7	3.6	4.0	6.7	5.1	6.0	12.4
II	4.8	4.4	4.0	4.5	5.3	5.7	5.8	3.8	4.8	5.5	5.0	5.2	9.2
III	4.7	5.7	5.2	5.0	5.0	5.7	5.4	4.2	4.8	7.0	6.0	5.9	3.2
IV	3.5	3.7	3.5	4.0	5.4	4.6	6.0	3.7	4.5	7.7	6.8	9.4	14.5
Total	17.2	18.8	18.1	18.4	20.0	21.2	23.9	15.3	18.1	26.9	22.9	26.5	39.3
Ave.	4.3	4.7	4.5	4.6	5.0	5.3	6.0	3.8	4.5	6.7	5.7	6.6	9.8

Analysis of variance

S.V.	D.F.	M.S.	COMP. F	PROB.
Temperature	4	15.25	5.77	<.01
Airdried vs. bindried	1	6.38	2.42	>.05
Among bindried	3	18.21	6.90	<.01
Depth	2	17.13	6.49	<.01
Depth × bindried T	6	2.82	1.07	<.05
Error	39	2.64		
Total	51			

	H.S.D.	
	.05	.01
Depth	1.41	1.79
Temperature	2.32	1.90

THE ANTHELMINTIC EFFICIENCY OF HYGROMYCIN IN SWINE[1]

G. Garcia, D. de Leon and M. Supnet

WITH TWO TEXT FIGURES

Hygromycin effectively controlled large roundworms and nodular worms but failed to improve feed efficiency.

The cost to produce a kilogram gain in weight was ₱0.73 for the control lot and ₱0.74 for the treated lot excluding cost of Hygromycin.

Hog raisers can avail themselves of two methods in combating internal parasites in swine. One is good sanitation, aimed at breaking the life cycle of the parasites. Spindler (1942) concluded that this is the cheapest way but, owing to some inherent disadvantages, it can be followed consistently by only a few hog raisers. Another is the use of appropriate deworming drugs after a correct diagnosis has been established. Supperer, as cited by Halama (1958), claimed this to be the only effective remedy against intestinal parasites in swine. Most deworming drugs, however, are toxic and have to be administered sparingly and carefully.

An anthelmintic which is claimed to be effective and safe to use in rations for pigs is Hygromycin.[2] Hahnel (1958) reported that Hygromycin, fed to pigs continuously, destroyed large roundworms, nodular worms, and whipworms before they could become mature and be able to lay eggs. According to McGowan *et al.* (1959), Hygromycin possesses anthelmintic effects against ascaris and *Oesophagostomum* spp.

To determine the anthelmintic efficiency of Hygromycin and the effects of parasitic load on the gain in weight and feed efficiency of pigs, this study was conducted from November, 1958, to July, 1959 in the Department of Animal Husbandry, U.P. College of Agriculture.

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[1] Central Experiment Station Contribution No. 2043.

[2] Supplied through the courtesy of Eli Lily, Pan American Corporation, Indiana, U.S.A.

MATERIALS AND METHODS

Twelve pigs (Berkshire and crossbreds) were grouped into two lots of six pigs each according to age, sex, breed and degree of ascarid infection with the least possible difference in egg count. Except for the ration, both lots received approximately identical care and management. The animals were weighed individually at the beginning of the study and every other week thereafter.

The College standard swine ration no. 2 was used as basal ration for both lots. Lot I (control) was fed just the basal ration; lot II (experimental) received Hygromycin which was thoroughly mixed with the ration at the rate of 2.26 kilograms per 1,000 kilograms of feed. The constituents of the ration, all parts by weight, are as follows:

Ground corn	15
Fine rice bran	60
Copra meal	18
Fish meal	7
Mineral mixture [3]	2
Total	102

The pigs were fed the amount they could readily consume in about 20 minutes twice daily, between 6:00 to 7:00 a.m. and 4:00 to 5:00 p.m. Great care was exercised to avoid unnecessary waste of feed and to prevent underfeeding. The animals were bathed every morning after feeding and given fresh drinking water at all times. Since the pigs were not allowed to pasture, soilage consisting mainly of sweet potato vines was given twice a week.

At the start of the study and every week thereafter, 10-gram fecal samples were collected from each animal in the morning and following Whitlock's Practical Dilution Egg-Count Procedure, the initial egg count was determined.

Each fecal sample was broken into small particles and a thick fecal suspension was made. Water was added up to the 150-cc. mark of a 250-cc. sampling bottle and the thin suspension thus formed was vigorously stirred. Approximately 10 cc. of the fecal suspension was poured through a 1-mm wire mesh into a small beaker. After working with one sample, the strainer was washed thoroughly to prevent contamination with the eggs in the previous samples.

[3] Composed of equal parts of finely ground shell and common table salt.

Exactly 0.5 cc. of the strained suspension was drawn into a graduated tuberculin syringe. An equal amount of saturated sugar solution made up of one pound granulated sugar dissolved in 12 ounce water and one per cent phenol as preservative, was added.

An air bubble large enough to considerably agitate the fecal suspension when moved up and down the syringe barrel was drawn into the syringe. After 0.2 cc. was expelled from the syringe as waste, three 0.15-cc. samples were expelled from the syringe and placed in the special glass slides prepared by glueing with balsam two of the small pieces of a broken slide to the ends of an ordinary glass slide, and attaching another glass slide to these supports to form a chamber for the smear. The smears were then examined under the microscope; the upper surface of each of the three samples was systematically scanned and the number of eggs from each sample was recorded. Three counts were made for every sample. The average number of eggs per sample was determined and multiplied by a factor, 200, to get the number of eggs per gram of feces.

The syringe was rinsed thoroughly with clean tap water after each use. Feces from both lots were collected everyday for 52 weeks and examined for the presence of expelled worms.

At the end of the first four months, three pigs from each lot were slaughtered. The same was done to the rest four months later. In both cases the intestinal tract of each animal was examined for roundworms and nodular worms.

RESULTS AND DISCUSSION

Ova Count (*Ascaris*). The initial egg count was higher by 356 EPG (eggs per gram) in the treated lot than in the control. A week after the start of the trial, a sharp decrease in the egg count was shown by the Hygromycin-fed pigs. The count was reduced to zero on the sixth week, indicating the anthelmintic properties of the antibiotic in destroying adult worms. In contrast, the ascarid egg count in the control lot, although variable, remained high throughout the study (fig. 1).

Ova Count (*Oesophagostomum*). The initial egg count was also higher in the treated lot than in the control, the difference being 1,766 EPG. A marked decrease in the egg count was noted in the Hygromycin-fed pigs on the second week; this was reduced to zero at about the 16th week of medicated feeding. A zero count was

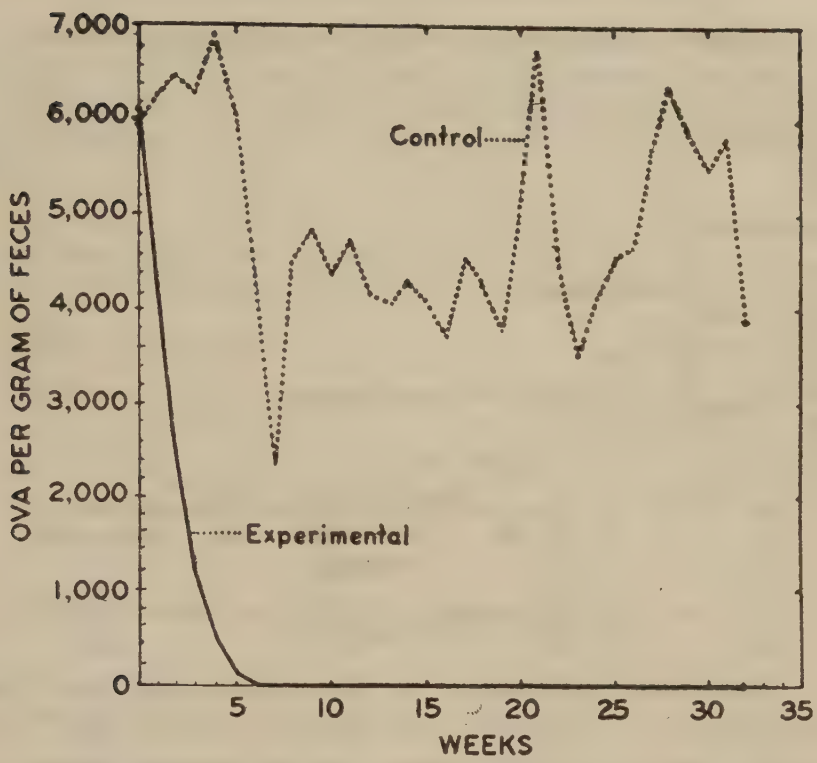

Fig. 1.—The effect of Hygromycin on ova counts of ascaris (large roundworm).

also obtained in the untreated pigs on the 30th week (fig. 2). Deyoe and Erider (1950) postulated that pigs became more resistant, had fewer worms and, as they grew older, suffered less from both the young worms in the lungs and the older worms in the intestines. Halama (1959) suggested that with increasing age and weight, the pig managed to control parasitic infestation to such a degree that a certain equilibrium between virulence of worms and resistance of the host developed as a result of antigen-antibody reaction.

Worms recovered in the feces. Due to the sterilizing effects of Hygromycin, adult ascaris continued to be voided out in the feces up to the 16th week in the treated animals although the egg count was reduced to zero on the sixth week. During the entire feeding period, 60 large roundworms were recovered in the feces of the treated lot as compared to only 10 in the control (fig. 1). Because of the difficulty of separating nodular worms from undigested food materials, the worms were not counted.

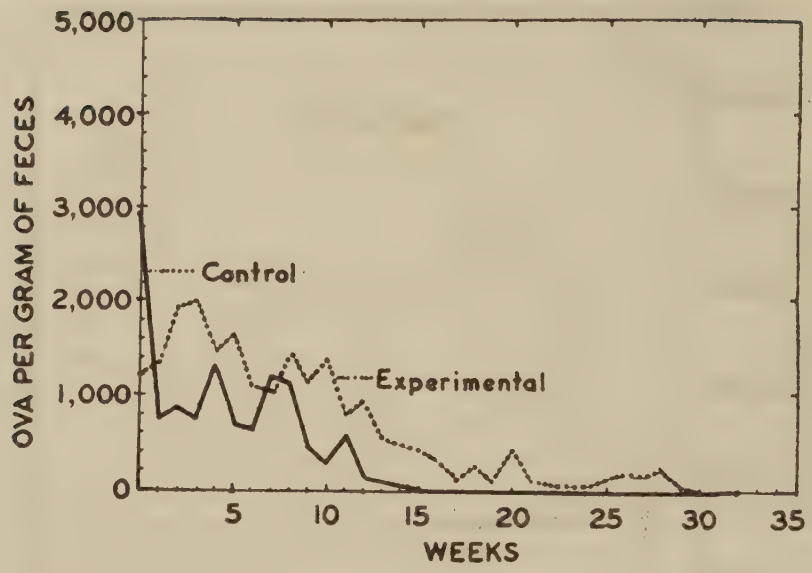

Fig. 2.—The effect of Hygromycin on weekly ova counts of nodular worms (Oesophagostomum).

Worms recovered at slaughter. The parasitic load was almost reduced to zero in the treated pigs (table 1) although pig no. 534

TABLE 1.—*Number of worms recovered from the animals at slaughter*

ITEMS	CONTROL ANIMAL NO.					
	561	563	553	533	555	530
Days tested	232	119	240	246	111	117
Number of parasites recovered:						
a) Large roundworms	7	27	17	7	7	2
b) Nodular worms	—	+[a]	—	—	+	+
c) Kidney worms	0	0	0	0	0	0

TABLE 1—continued

	TREATED ANIMAL NO.					
	531	557	556	534	559	560
Days tested	117	111	232	246	119	240
Number of parasites recovered:						
a) Large roundworms	0	0	0	1	0	0
b) Nodular worms	—	—	—	—	—[b]	—
c) Kidney worms	0	5	0	0	3	0

[a]—Positive.
[b]—Negative.

harbored on immature ascarid worm at necropsy. Two pigs had kidney worms. On the other hand, 67 large roundworms were found in the control pigs. Nodular worms were found in the untreated pigs slaughtered after four months; none was found in those killed later.

Rate of growth. The control pigs and the treated pigs made an average daily gain of 0.45 and 0.36 kilogram, respectively. (table 2). The average final gain in weight was higher by 12.2 kilo-

TABLE 2.—*Total gain in weight in kilograms of the pigs for the entire 35-week test*

CONTROL LOT		TREATED LOT	
Animal Number	Gain in Weight	Animal Number	Gain in Weight
561	121.5	531	36.4
563	32.2	557	33.4
533	96.6	534	87.6
555	55.0	559	32.5
530	50.6	560	107.9
553	119.8	556	103.3
Total	475.5	Total	401.1
Mean	79.1	Mean	66.9

Analysis of variance

SV	DF	SS	MS	F	PROBABILITY
Among rations	1	461.28	461.28	0.32	>.05
Pigs within rations	10	14,006.41	1,400.64		
Total	11	14,467.69			

grams in the control probably because of the difference in the weaning and initial weights between the two lots. The mean difference in weaning weight was 1.2 kilogram and in initial weight, 7.5. kilograms in favor of the untreated pigs. The heavier weight results in the control lot agreed with the findings of Thrasher *et al.* (1958) and of Hoefer and Luecke (1958) who observed that Hygromycin did not stimulate growth of worm-free pigs. However, in similar studies, Conrad and Besson (1958) reported that during the first 59 days the addition of the antibiotic had no effect on gains in unwormed pigs but increased gains by nine per cent in wormed pigs. During the second 59 days (118 days) Hygromycin significantly

depressed gains and reduced feed intake, resulting in more feed needed per 100-pound gain.

Food consumed. Although the Hygromycin-fed animals consumed 269.6 kilograms of concentrates less than the control, the amount of feed per kilogram of gain in weight was almost the same for both lots, the control utilizing 4.0 kilograms and the treated lot, 4.1 kilograms (table 3). The difference in feed efficiency agreed

TABLE 3.—*Summary of results*

ITEMS	CONTROL	TREATED
Number of pigs per lot	6	6
Average initial weight, kg.	27.2	19.7
Average final weight, kg.	106.3	86.5
Average total gain, kg.	79.1	66.9
Average daily gain, kg.	0.4	0.4
Feed per kg. gain, kg.	4.0	4.1
Feed cost per kg. gain, ₱	0.73	0.74 *
Number of roundworms voided	10.0	60.0
Number of roundworms recovered	67.0	1.0
Roundworms:		
Average ova count (initial), EPG	957.0	1,016.0
Average ova count (final), EPG	1,310.0	
Nodular Worms:		
Average ova count (initial), EPG	200.0	494.0
Average ova count (final), EPG	0	0

* Cost of Hygromycin not included.

with the findings of Conrad and Besson (1958) for confined unwormed pigs but contradicted those for both the unwormed pigs and those previously wormed on pasture. The cost to produce a kilogram gain in weight was higher by ₱0.01 in the treated pigs excluding cost of the antibiotic.

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THE VALUE OF COARSE BREWER'S SPENT GRAINS AS A FEED SUPPLEMENT FOR DAIRY HEIFERS [1]

C. M. Demerre, L. E. Nazareno and C. B. Perez, Jr.

WITH FIVE TEXT FIGURES

The chemical composition of the coarse brewer's spent grains used in this study is as follows: protein, 15.40 per cent; fat, 3.80 per cent; fiber, 18.05 per cent; nitrogen-free-extract, 48.70 per cent. Digestibility values of 11.57 per cent protein and 56.61 per cent T.D.N. were obtained from digestion trials.

In a separate feeding experiment, the heifers in treatment II (coarse brewer's spent grains-molasses mixture as partial replacement for soilage) consistently showed better performance than those in treatment I (full soilage) when gains in body weight, height at withers, and circumference at heart girth were used as criteria for growth. The difference in increment in heart girth in favor of treatment II was highly significant.

Under the conditions of this study, coarse brewer's spent grains or coarse brewer's bagasse plus molasses appears to be a satisfactory feed supplement. It is a low-protein concentrate that also supplies the bulk essential in ruminant feeding.

The inadequate supply of green roughage in summer is a common problem among livestock raisers in the Philippines. The need is felt even more sharply by the few dairymen in our country. Very often, rice straw, which has poor feeding value, is used to supplement whatever amount of soilage is available. In a few cases, corn and grass silages are used. However, silage production demands more time, effort, and money from the farmers.

After the heifer is past the most critical period of life, i.e., during the first six months, the tendency is to rough her through with very little care. However, it should be understood that heifers cannot grow normally unless they receive an adequate supply of total digestible nutrients.

Dairy heifers should be fed in such a way that they will make good gains, so that they may obtain their full size (Henderson and

[1] Central Experiment Station Contribution No. 2044.

Reaves, 1954). Morrison (1956) stated that the necessity of feeding grains and other concentrates depends on the nature of the pasture. On an average pasture, only a small amount of concentrates is necessary to maintain good growth. But on a scanty pasture, it is important to provide plenty of other feed, including concentrates, hay, silage, and green soiling crops.

Several replacement feeds have already been tried with some success. In the Philippines, Castillo *et al.* (1957) have shown that corncobs may be used in feeding Murrah heifers for short durations without obvious ill effects. From the wineries, by-product feed such as distiller's dried grains with and without solubles have proved valuable for dairy animals (Schabinger and Knodt, 1947; Elliott *et al.*, 1951; Slack and Turk, 1951; Loosli *et al.*, 1952; Loosli and Warner, 1953).

Henderson and Reaves (1954) classify brewer's grains, a residue from the brewing of barley in the making of beer, as a medium high-protein concentrate. Its average composition, as given by Morrison (1956), is 27.5 per cent protein, 6.5 per cent fat, 14.2 per cent fiber, and 41.1 per cent nitrogen-free-extract. Limited information is obtainable on the value of the coarser by-products of the beer industry as animal feeds. The spent hops, left after the wort has been boiled with hops and filtered, are sometimes dried and used for feed or fertilizer. They contain not only the hop residue, but also some protein precipitated from the wort in the boiling process. Davies and Sullivan (1929), as cited by Morrison (1956), said that the digestibility of the spent hop is so low that the product is of doubtful value as a feed. Morrison further claims that since the spent hops are bitter and unpalatable to the stock, only a small proportion can be included in a mixture of better-liked feeds.

Another by-product in the manufacture of beer is coarse brewer's spent grains (fig. 1), locally referred to as coarse brewer's bagasse. It is the residue (husk) left after the mealy endosperm has been completely disintegrated during the milling of the malt. This is produced in commercial quantities and is becoming popular among horse owners in Manila and suburbs. It is also used as an ingredient in the ration of dairy cattle by at least two dairymen in the country. This study was therefore conducted to determine the possible use of coarse brewer's spent grains as a partial replacement for green roughage for growing dairy heifers.

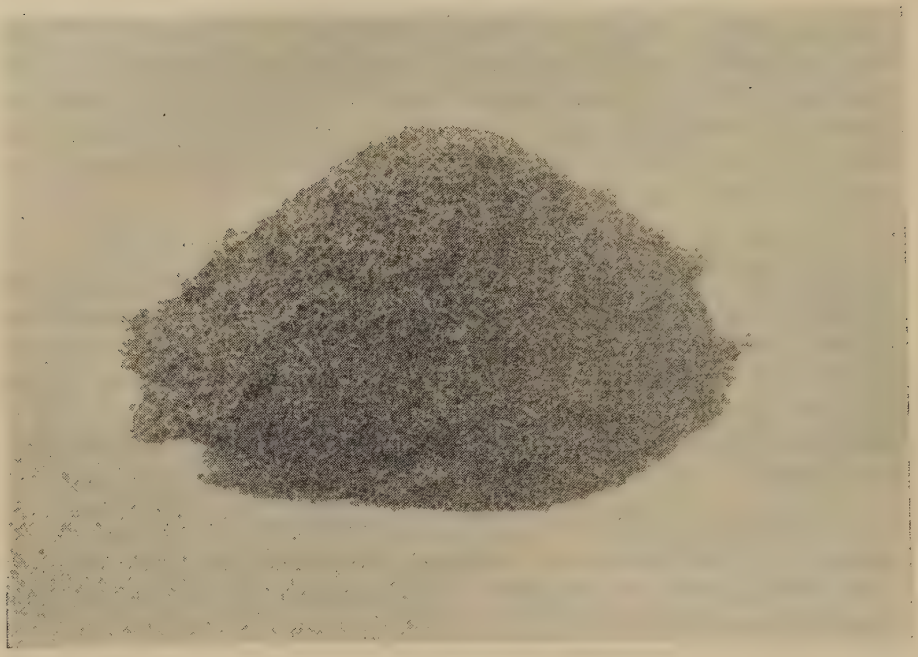

Fig. 1.—Coarse brewer's spent grains or coarse brewer's bagasse.

The study was divided into two work areas. The first was done to determine the digestibility of coarse spent grains, and the second, to determine the feeding value of the same feed as a partial replacement for soilage given to dairy heifers.

MATERIALS AND METHODS

Digestibility of coarse brewer's spent grains

Six grade Shropshire sheep were used in the digestion trials, the procedures of which were described by Loosli *et al.* (1954). To increase the palatability of the coarse spent grains, molasses was mixed, composing 10 per cent of the mixture. No concentrate feed was given; instead, para grass was fed to the animals.

Digestion coefficients for coarse spent grains were calculated by difference, using values actually determined by feeding para grass alone in a separate digestion trial and Morrison's coefficients for molasses.

Table 1 shows the chemical composition of coarse spent grains and the average digestion coefficients for the six grade Shropshire sheep. For comparison, a proximate analysis of the same feed done

at the laboratory of B-Meg Feed Products Plant is also shown.

TABLE 1.—*Average chemical composition and digestion coefficients of coarse brewer's spent grains*

	PROTEIN	FIBER	FAT	N-F-E
	per cent	*per cent*	*per cent*	*per cent*
Chem. composition	15.40	3.80	18.05	48.70
B-Meg analysis *	18.10	3.90	20.10	38.10
Digestion coeff.	75.1±3.2	64.1±15.3	44.2±8.2	64.7±6.5
Digestible nutrients	11.57	2.44	7.98	31.57

* Furnished by San Miguel Brewery B-Meg Feed Products Plant.

From the result of this study, coarse spent grains may be classified as a low-protein concentrate. Its digestibility is low. The coarse spent grains contain 11.57 per cent digestible protein and 56.61 per cent total digestible nutrients.

Feeding value of coarse spent grains

Ten growing dairy heifers selected on the basis of age, weight, and blood composition were evenly distributed into two groups. Five heifers were assigned at random to treatment I (full soilage) and five to treatment II (coarse spent grains-soilage ration).

Two test periods of 84 days each, preceded by 14 days of preliminary adjustment, were made. Each heifer was confined in an individual pen adequately provided with a feed box and a water trough. The extension runs were littered with sawdust to provide comfort to the animals and improve drainage. Fresh water was made available to each animal at all times.

Both treatments received the same concentrate mixture consisting of the following ingredients, all parts by weight:

Rice bran	35.0 kg.
Corn gluten feed	20.0 "
Copra meal	20.0 "
Ground yellow corn	15.0 "
Soybean oil meal	8.0 "
Salt	1.0 "
Ground oyster shell	0.5 "
Bone meal	0.5 "
Total	100.0 "

The mixture contained 13.46 per cent digestible protein and 70.65 per cent total digestible nutrients. On the average, each animal in both lots received a daily allowance of 1.6 kilograms of the mixture.

Soilage was given in large amounts to each heifer in the control lot to enable the animal to eat as much as it could and leave some as refuse. In the experimental lot, however, the amount of soilage given was reduced in variably increasing proportions, based on the average consumption of the five heifers in the control lot during the previous week. As replacement for soilage withheld from the animals, the coarse spent grains-molasses (90:10) mixture was given in amounts that when added to the concentrate and limited soilage intakes would furnish the adequate amount of T. D. N. in the entire ration for the animals. To enable the heifers in the experimental lot to eat more of the coarse spent grains-molasses mixture, a part of each day's ration was mixed with the concentrate mixture in variable proportions. The remaining portion of the coarse spent grains-molasses mixture was fed separately.

The average weight, height at withers, and circumference at heart girth of each heifer were taken at the same time in the morning for three successive days and recorded as the initial data. In addition, measurements were taken once every 28 days. The former procedure was followed at the end of the 84-day period to get the final data. The differences between the initial and final weights, heights, and circumferences were used as criteria for determining the relative feeding value of coarse spent grains and soilage.

RESULTS AND DISCUSSION

At the start of the study the average initial age of the heifers in treatment I was 11.4 months, and that in treatment II, 11.5 months. Treatment I had initial measurements of 147.2 kilograms weight, 104.1 centimeters height at withers, and 120.2 centimeters circumference at heart girth; treatment II had 147.7 kilograms, 101.6 centimeters, and 121.5 centimeters.

Live weight. At the end of the first 84-day comparison period, the average gain in weight of the heifers in treatment I was 19.3 kilograms, and in treatment II, 19.5 kilograms. The corresponding daily gains in weight in treatments I and II were 0.230 and 0.232 kilogram, respectively. In the second period, the heifers in treatment I gained 27.3 kilograms while those in treatment II gained 42.5 kilograms. The corresponding daily gains were 0.325 and 0.506 kilogram. While the

animals in both treatments made almost identical gains in the first period, those given the coarse spent grains-molasses supplement in the second period showed more gains.

The individual gains in weight of both treatments for the two periods are summarized in table 2. On the basis of weight, there were no significant differences in the rate of growth between the animals in treatments I and II. The higher daily gains in weight of the animals in treatment II may be due to the fact that the total amount of feed consumed by these animals contained slightly more digestible nutrients than the total feed consumed by those in treatment I.

It may be mentioned that the heifers at the start of the study were in depleted condition owing to tick infestation and to the system of management followed before the animals were used in the experiment. Apparently, these factors accounted for the fairly low increase in weight of all animals during the first period. Tick infestation was, however, controlled by regular spraying with 0.03 per cent lindane solution. Figures 2a, 2b, and 3a, 3b show the conditions of the animals at the end of the first and second periods, respectively. The greatly improved conditions attained by the animals in both lots at the end of the second 84-day comparison period affirm the opinion of Waters (1908) and Eckles and Sweet (1919) that an animal may be temporarily stunted and yet reach the normal size of the breed if later placed under favorable conditions.

An interesting observation was noted in the case of heifers No. 604 and No. 606 (fig. 4a, 4b, 5a, 5b). At the start of the study, the former, assigned to treatment I, had an initial weight of 165.0 kilograms while the latter, in treatment II, weighed 156.3 kilograms. Their final weights were 183.7 and 191.0 kilograms, heifer No. 606 of treatment II being heavier by 7.3 kilograms. When the treatments were reversed in the second period, heifer No. 604 (now in treatment II) had a final weight of 245.0 kilograms, while heifer No. 606 weighed only 221.0 kilograms. In both instances, the heifers supplemented with coarse spent grains-molasses mixture made a greater gain in weight in spite of their low initial weight in each period.

Growth of the heifers in terms of body weight in both treatments in either period is similar to that reported by Castillo *et al.* (1956) for Holstein-Red Sindhi grades of similar ages. The average daily gain in weight of 0.51 kilogram per animal among those sup-

TABLE 2.—*Gain in weight, increase in height at withers, and increase in circumference at heart girth during the first and second 84-day periods*

TREATMENT		1	2	3	4	5	6	7	8	9	10	TOTAL	MEAN
Weight	I	25.7	29.0	32.3	16.7	32.7	12.6	32.0	7.3	18.6	25.7	232.6	23.3
(kg.)	II	34.7	12.7	17.0	19.0	14.3	34.0	60.7	14.7	59.0	44.6	310.7	31.1
Height at	I	5.4	1.5	4.5	6.2	3.5	1.8	4.2	1.8	2.8	3.0	34.7	3.5
withers (cm.)	II	5.0	7.0	4.3	3.0	3.5	4.0	6.8	5.6	7.3	2.6	48.9	4.9
Circumference at	I	4.2	4.3	4.0	6.3	6.5	3.8	6.3	5.5	7.5	5.6	54.0	5.4
heart girth (cm.)	II	10.2	3.2	9.0	9.6	5.5	9.0	9.5	6.5	12.7	12.0	87.2	8.7

ANALYSIS OF VARIANCE

Weight

S.V.	d.f.	MS	F
Treatments	1	304.98	1.44
Within treatments	18	212.13	
Total	19		

Height at withers

S.V.	d.f.	MS	F
Treatments	1	10.08	3.63
Within treatments	18	2.77	
Total	19		

Heart girth

S.V.	d.f.	MS	F
Treatments	1	55.11	10.96
Within treatments	18	5.03	
Total	19		

Fig. 2a.—Control lot. Grade heifers at the end of the first 84-day period.

Fig. 2b.—Experimental lot. Grade heifers at the end of the first 84-day period.

Fig. 3a.—Control lot. Grade heifers at the end of the second 84-day period.

Fig. 3b.—Experimental lot. Grade heifers at the end of the second 84-day period.

Fig. 4a.—Grade heifer No. 604 in the control lot at the end of the first 84-day period. (Initial weight, 165.0 kg; final weight, 183.7 kg.).

Fig. 4b.—Grade heifer No. 606 in the experimental lot at the end of the first 84-day period. (Initial weight, 156.3 kg; final weight, 191.0 kg.).

Fig. 5a.—Grade heifer No. 606 in the control lot at the end of the second 84-day period. (Initial weight, 195.3 kg; final weight, 221.0 kg.).

Fig. 5b.—Grade heifer No. 604 in the experimental lot at the end of the second 84-day period. (Initial weight, 186.0 kg.; final weight, 245.0 kg.).

plemented with coarse spent grains-molasses mixture is comparable to Morrison's (1956) values of 1.16 pounds for Guernseys and 1.20 pounds for Jerseys at one year of age.

Height at withers. Since the fleshy part of the animal does not increase at the same rate as the skeleton, it can be seen that growth cannot be measured by weight alone. Eckles and Anthony (1956) suggested that the height at withers would be the easiest and the most satisfactory measure of the growth of the skeleton.

The heifers in treatment I and treatment II made an average increase in height at withers of 2.7 and 4.5 centimeters at the end of the first period, or a corresponding average daily increase of 0.032 and 0.053 centimeters, respectively. A similar trend of growth followed in the second 84-day comparison period when the treatments were reversed. At the end of the second period, the average increase in height of the heifers in treatment I was slightly less than that obtained by the heifers in treatment II. The former made an average daily increase in height at withers of 0.050 centimeter, as against 0.062 centimeter of the latter.

The data on individual increase in height at withers of both treatments for the two periods are also summarized in table 2. The difference in mean increase in height at withers of the animals in treatments I and II was not significant.

Heart girth. The heifer's average increase in circumference at heart girth during the first 84-day comparison period was 5.7 centimeters in treatment I and 7.5 centimeters in treatment II indicating individual daily increases of 0.068 centimeter and 0.089 centimeter, respectively. When the treatments were reversed in the second period, a much greater increase was observed among the heifers in treatment II. While the heifers in treatment 1 had an individual average daily increase of only 0.032 centimeter, those in treatment II had an increase of 0.117 centimeter.

Table 2 shows the individual increase in heart-girth measurement for each treatment in both periods. The difference in heart girth measurement between the two treatments was highly significant ($P<0.01$).

Feed consumption. Soilage fed to the animals was composed largely of para grass. The calculated nutrients from 1.0 kilogram of each feed given were as follows:

	D.P.	T.D.N.
Concentrate mixture	0.13	0.71
Brewer's spent grain mixture	0.12	0.56
Para grass	0.01	0.10

Digestible protein and total digestible nutrients for concentrate, brewer's spent grain mixture, and para grass soilage were estimated, using data obtained by Morrison (1956), and Loosli, *et al.* (1954); and from this study.

The average amount of feed consumed daily by each heifer fed full soilage was 11.7 kilograms during the first 84-day period, while each heifer supplemented with coarse brewer's spent grains consumed 9.4 kilograms (table 3). In the second period, the average total feed consumption of the heifers in soilage lot was 15.3 kilograms, whereas that of the heifers in the brewer's spent grain-lot was 9.2 kilograms.

TABLE 3.—*Average daily consumption per heifer, in kilograms*

COMPARISON PERIOD	TREATMENT I (full soilage)			TREATMENT II (partial soilage)			
	Concentrates	Soilage	Total	Concentrates	C.B.[a] S.G.	Soilage	Total
1	1.6	10.7	11.7	1.6	1.0	6.8	9.4
2	1.6	13.7	15.3	1.6	2.0	5.6	9.2

[a] Coarse brewer's spent grains.

Soilage consumption of the coarse brewer's spent grain lot during the first period was greater than during the second period by 1.2 kilograms. The treatment II heifers' tendency to consume more soilage during the first period is attributed to the fact that the animals did not readily take to the coarse brewer's spent grains-molasses mixture. After having been conditioned to the ration, however, the animals relished the feed supplement. This is shown by an average increase, during the second period, of 1.0 kilogram in the amount consumed. Coarse brewer's spent grains replaced 32.71 and 59.12 per cent of the soilage during the first and second periods, respectively.

SUMMARY AND CONCLUSION

1. Proximate analysis of coarse brewer's spent grains conducted at the Nutrition Laboratory of the Department of Animal Husban-

dry gave the following composition: protein, 15.40 per cent; fat, 3.80 per cent; fiber, 18.05 per cent; nitrogen-free-extract, 48.70 per cent.

2. Digestion coefficients for the coarse brewer's spent grains were calculated by difference, using values actually determined by feeding para grass alone in a separate digestion trial and Morrison's (1956) coefficients for molasses. Coarse brewer's spent grains were calculated to contain 11.57 per cent digestible protein and 56.61 per cent total digestible nutrients.

3. In a feeding trial, 10 Holstein-Red Sindhi grades of growing dairy heifers were divided into two groups of approximately equal initial weights. Five heifers were assigned at random to treatment I (full soilage) and five to treatment II (coarse brewer's spent grain-soilage ration). Two test periods of 84 days each, preceded by 14 days of preliminary adjustments, were made. The treatments were reversed at the end of the first period, using the same set of animals.

4. At the end of the first 84-day comparison period, the daily gains in weight of the heifers in treatments I and II were 0.230 kilogram and 0.232 kilogram, respectively. In the second period, treatment I had an average daily gain of 0.325 kilogram as against 0.506 kilogram in treatment II. While the animals in both treatments made almost identical gains in the first period, more gain was obtained by those supplemented with coarse brewer's spent grains-molasses mixture at the end of the second period. Individual gains in weight between the two treatments for the two periods did not differ significantly.

5. The heifers in treatment I and treatment II made average increases in height at withers of 2.7 and 4.5 centimeters, respectively, during the first period. In the second period, however, the heifers in treatment I had an average increase in height at withers of 4.2 centimeters, while those in treatment II had 5.3 centimeters.

6. The difference in heart-girth measurement, in favor of treatment II for the two periods, was highly significant.

7. Under the conditions of this study, coarse brewer's spent grains or coarse brewer's bagasse plus molasses appears to be a satisfactory feed supplement. The digestibility of coarse brewer's spent grains is relatively low when compared to other concentrates, but certainly higher than most of the locally available green roughages. Furthermore, coarse brewer's bagasse supplies the bulk essential in ruminant feeding.

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PRELIMINARY STUDIES ON THE PRESERVATION OF BOVINE AND BUBALINE SEMEN AT ROOM TEMPERATURES[1]

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The IVT-extended semen of bulls and Murrah bulls with 50 per cent motility lasted about four days at room temperature and the yolk-citrate semen about five days in the refrigerator.

With 30 per cent motility, the IVT-extended semen lasted for only seven days, whereas the refrigerated, from nine to ten days.

The preservation of good quality semen at room temperature is very important especially in areas where electricity and refrigeration units are not available. The Philippines has many such areas. The maintenance of the high fertility of bull semen at ordinary room temperature for several days will make possible the rapid and flexible application of artificial insemination and faster and more economical improvement of our farm animals.

Anderson (1945) showed that spermatozoa stored at 5°C. survived longer than when stored at higher temperatures. Foote and Salisbury (1948) reported that the motility of spermatozoa stored at 20°C. in a citrate-phosphate buffer was prolonged by the addition of a number of antibacterial agents. Foote and Bratton (1950) found that after 72 hours of storage, the percentage of motile spermatozoa stored at 25°C. was lower even with the combination of sulfanilamide and antibiotic than that stored at 5°C.

In a preliminary investigation at the University of Illinois, VanDenmark and Sharma (1957) reported that the fertility of the IVT-diluted bovine semen did not decrease during six to seven days' storage at room temperature of 65° to 80°F.; that the over-all non-return rate of 75.7 per cent was nine per cent greater than that for the same ejaculate in the ordinary yolk-citrate diluent stored for one to three days at 40°F. However, Dunn and Foote (1957) contradicted these by reporting that the non-return rates for IVT-extended semen stored at room temperature were: first day, 69; second day, 45; third day, 33; fourth day, 26; fifth day, 25; and

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sixth day, 21 per cent. The average non-return rate for 838 total services was 38 per cent. High ambient temperatures, defects in ampules and the two-hour processing procedure contributed to the low fertility.

Because of the controversial results among previous workers and the differences in climate and other factors, this study, aimed at determining the possibility of preserving the quality of bovine semen at room temperature and at different ages, was undertaken at the Department of Animal Husbandry, U.P. College of Agriculture, from July, 1957 to July, 1958.

MATERIALS AND METHODS

Three mature bulls [2] of different breeds (Sta. Gertrudis, Brown Swiss, and Red Sindhi), a one-and-one-half year old Holstein-Freisian bull, and five mature Murrah bulls [3] were used. The Holstein, Brown Swiss and Red Sindhi bulls and Murrah bull nos. 22 and 24 were from the College herd; the Sta. Gertrudis bull and Murrah bull nos. 1, 6, and 3099 from the Bureau of Animal Industry herd. All these animals were apparently healthy.

Semen was collected from these animals by the use of the artificial vagina at regular intervals of seven days. The quality of five ejaculates taken at random from each animal was evaluated immediately after collection. The motility of the fresh semen and the morphology of the sperms were determined through the microscope. The semen samples were normal with respect to the proportion of defective to normal spermatozoa. There was no marked difference between the percentage of abnormal sperms in the bull semen (11.40 per cent) and Murrah bull semen (11.68 per cent), and between the concentration of bull and murrah bull semen as determined with the photolometer. Dead-alive staining was performed using an eosin-aniline blue staining mixture formulated by Shaffer and Almquist (1948).

A diluent was prepared by heating to near boiling 20.0 gm. sodium citrate dihydrate, 2.1 gm. sodium bicarbonate, 0.4 gm. potassium chloride, 3.0 gm. glucose, and 3.0 gm. sulfanilamide in a liter of water redistilled over glass. The solution was cooled to room temperature and was saturated with carbon dioxide by bubbling the

[2] In this study "bull" refers to *Bos taurus* and *Bos indicus*.
[3] "Murrah bull" in this experiment refers to *Bobalus bubalis*.

gas in it for 10 minutes or until the pH was reduced to about 6.35. After saturation, 1,000 I.U. penicillin and 1,000 mg dihydrostreptomycin sulfate per ml. of the diluent, and a sufficient volume of fresh egg yolk to make a final diluent of 10 per cent egg yolk were added. This is termed by VanDenmark and Sharma (1957) as the Illini Variable Temperature (IVT) diluter.

A commonly used yolk-citrate diluent consisting of equal parts egg yolk and a 2.9 per cent solution of sodium citrate dihydrate with 0.6 gm. sulfanilamide and antibiotics (500 I.U. penicillin and 500 UG streptomycin per ml. of diluent) was also prepared.

Using the split ejaculate technique, one portion of the semen was diluted with IVT diluent so that each ml. contained approximately 25 million sperms. One ml. aliquots of the diluted semen were sealed in glass ampules (1.2 ml. total capacity) and stored in the dark at room temperature (75° to 95°F).

The other portion, used as the control, was diluted with the yolk-citrate diluent to a concentration of 25 million sperms per ml., cooled gradually by immersing in cool tap water for one hour, and then stored at 40°F in accordance with the standard practice.

The effectiveness of each diluter in maintaining the motility of the sperm was compared. Analyses and evaluation of the semen samples were made every 24 hours until the motility reading dropped to 30 per cent.

RESULTS AND DISCUSSION

The decrease in the percentage of live spermatozoa in bull and Murrah bull semen stored in IVT- and in yolk-citrate extenders was directly proportional to the decline in motility (table 1a). The vital staining showed that in addition to the motile sperms, 15 to 30 per cent of the non-motile sperms were alive. The writers' findings corroborate those of Lasley (1944) who reported that the percentage of live sperms as determined by the opal blue-eosin staining method was highly correlated with the percentage of progressively motile sperms. He further stated that a few minutes after collection, there was an average of 16.9 per cent dead sperms, 19 per cent non-motile live sperms, 12.5 per cent weakly motile and 51.9 per cent progressively motile sperms. After four days storage, the average of dead sperms was 27.7; of non-motile live sperms,

37.4; of weakly motile, 20.7; and of progressively motile, 14.2 per cent.

TABLE 1a.—*The relationship between motility and vital staining*

LENGTH OF STORAGE	BULL				MURRAH BULL			
	Motility		Vital staining		Motility		Vital staining	
	IVT	Y-C	IVT	Y-C	IVT	Y-C	IVT	Y-C
day				*per cent*				
Fresh	69.00	69.00	80.96	80.96	68.40	68.40	81.48	81.48
1	67.00	67.00	79.12	79.44	66.80	66.00	79.99	79.60
2	61.75	62.50	75.05	75.73	61.80	61.60	75.65	76.47
3	55.75	57.00	70.12	71.57	56.60	58.40	71.16	73.69
4	48.50	52.00	63.79	67.37	50.20	53.40	64.85	69.11
5	42.25	49.75	56.88	64.59	44.40	49.40	58.88	65.74
6	38.25	44.75	52.28	59.32	40.23	46.09	54.73	62.53
7	32.50	40.50	46.67	55.44	37.31	42.39	53.43	48.92

The difference between the percentages of live and motile spermatozoa tended to increase with longer storage both for the IVT- and yolk-citrate-extended semen of bulls and Murrah bulls. This result conformed with the findings of Madden, Herman, and Berouseck (1947) that the motility slightly decreased with storage but more quickly than the percentage of live sperms; that the initial percentage of live sperms was significantly correlated with initial motility of semen but that variations in the percentage of live sperms in each motility class were wide.

The correlation between motility reading and vital staining both in IVT- and yolk-citrate-extended semen of bull and Murrah bull was high (table 1b). All correlation values was significant at one per cent probability level, indicating that the motility reading was a good measure of the proportion of live sperms.

TABLE 1b.—*The correlation*[a] *between motility and vital staining*

LENGTH OF STORAGE	BULL		MURRAH BULL	
	IVT	Y-C	IVT	Y-C
day				
Fresh	.873	.873	.701	.701
1	.894	.899	.781	.824
2	.947	.923	.927	.903

[a] Significant at one per cent probability level.

The difference in the length of storage above 50 per cent motility level between IVT- and yolk-citrate-extended semen in Red Sindhi and in Sta. Gertrudis bulls was not significant (table 2). The 50 per cent motility of Sta. Gertrudis bull semen was maintained for about three days under both treatments; whereas, that of the Red Sindhi, for about five days. This might be explained by the fact that all the ejaculates obtained from the Sta. Gertrudis bull had an initial motility of 60 per cent; whereas those from the Red Sindhi bull had from 65 to 85 per cent.

TABLE 2.—*Average number of days extended bull semen maintained a minimum of 50 per cent motility*

BULLS	TREATMENT		MEAN DIFFERENCE	HSD	
	IVT	Y-C		0.05	0.01
	day	*day*	*day*		
Red Sindhi	4.6	5.0	0.4	1.5057	2.0267
Sta. Gertrudis	3.4	2.6	0.8		
Brown Swiss	3.0	5.4	2.4 [a]		
Holstein	3.8	6.4	2.6 [a]		
Mean	3.70	4.85			

[a] Significant at one per cent probability level.

The difference between the effect of IVT- and of the yolk-citrate extender on the length of storage of split ejaculates of the Brown Swiss and the Holstein bull semen was significant at the one per cent level. The yolk-citrate extender maintained the 50 per cent motility level of the semen longer than the IVT extender. However, the difference in the length of storage of an IVT-diluted semen between the Holstein and the Brown Swiss was not marked, and in the storage of the yolk-citrate-treated semen, the difference was only one day in favor of the Holstein.

The Red Sindhi and Sta. Gertrudis semen with about 50 per cent motility, could be stored under room temperature (75° to 95°F) in an IVT extender for three to five days. This length of time was also observed on the yolk-citrate-refrigerated samples. On the other hand, the Brown Swiss and Holstein bulls' semen in the IVT extender was kept successfully from three to four days at room temperature; whereas that in the refrigerated yolk-citrate diluter, from five to six days.

However, for cattle in general, the differences in the mean average length of time the IVT and yolk-citrate samples maintained the 50 per cent motility were not significant. The mean average of the semen samples under IVT-room temperature extender was one day less than that in the yolk-citrate-regrigerated extender.

The yolk-citrate treatment of the Red Sindhi and the Sta. Gertrudis semen maintained 30 per cent motility two days longer than the IVT treatment (table 3).

TABLE 3.—*Average number of days extended bull semen maintained a minimum of 30 per cent motility*

BULLS	TREATMENT		MEAN DIFFERENCE	HSD	
	IVT	Y-C		0.05	0.01
	day	*day*	*day*		
RedSindhi	7.8	9.4	1.6	2.5085	3.3765
Sta. Gertrudis	6.0	8.2	2.2		
Brown Swiss	7.0	10.0	3.0 [a]		
Holstein	6.4	10.2	3.8 [b]		
Mean	6.80	9.45			

[a] Significant at five per cent probability level.
[b] Significant at one per cent probability level.

t-table

TREATMENT	MEAN	COMPUTED *t*
	day	
IVT	6.80	6.16 [a]
Y-C	9.45	

[a] Significant at one per cent probability level.

Statistical analysis, however, showed that this difference was not significant. The Brown Swiss semen and the Holstein semen treated with yolk-citrate stored three and four days longer, respectively, than that treated with IVT diluter. These differences are statistically significant. In general, bulls' semen treated with IVT retained above 30 per cent motility for about six to seven days and that with yolk–citrate, eight to 10 days.

An IVT-extended bull semen was stored for about three to four days at room temperature, with good motility which progressively decreased after the fourth day, but was maintained at 30 per cent

to about the seventh day (tables 2 and 3). However, the refrigerated yolk-citrate treatment of this same semen sample maintained 50 per cent motility for about five days and 30 per cent motility for around nine days.

The difference in the length of storage between the IVT- and the yolk-citrate-extended semen of Murrah bulls 1 and 6 at 50 per cent motility level was not marked (table 4). Although the yolk-citrate-extended semen from Murrah bull 22 was stored one day longer, and that from Murrah bulls 24 and 3099, two days longer than IVT samples, the mean average storage time for these five Murrah bulls was statistically insignificant.

TABLE 4.—*Average number of days Murrah Buffalo-extended semen maintained a minimum of 50 per cent motility*

BULLS	TREATMENT	
	IVT	Y-C
	day	*day*
Murrah No. 1	4.6	5.0
Murrah No. 6	5.8	5.2
Murrah No. 22	3.2	4.6
Murrah No. 24	3.8	6.0
Murrah No. 3099	2.8	4.6
Mean average	4.04	5.08

The storage duration above 30 per cent motility level of semen samples extended with yolk-citrate and stored at 40°F was consistently longer than that of samples extended with IVT and stored at room temperature (table 5). The semen lasted for nine to 10 days in the refrigerated yolk-citrate extender and only about six to eight days in the IVT diluent kept at ordinary room temperature. The mean difference was significant at one per cent probability level.

Murrah Bull semen with motility of about 50 per cent was stored for about four days and that with 30 per cent, for about seven days at room temperature in an IVT extender (tables 4 and 5). These same semen samples diluted with a yolk-citrate diluter and stored in the refrigerator maintained 50 and 30 per cent motility for five and ten days, respectively.

For about four days, the IVT-extended bull semen stored at ordinary room temperature was just as good as the yolk-citrate-

treated semen stored in the refrigerator at 40°F. On the fifth day, the IVT-extended bull semen had a marked decline in motility, whereas the refrigerated semen had about 50 per cent motility which was still good for insemination. From the sixth day onward, the motility of the refrigerated semen dropped below 50 per cent. The motility decreased to about 32 per cent on the seventh day for the IVT-diluted semen and to about 35 per cent on the ninth day for the yolk-citrate-diluted semen.

TABLE 5.—*Average number of days Murrah Buffalo-extended semen maintained a minimum of 30 per cent motility*

BULLS	TREATMENT	
	IVT	Y-C
	day	*day*
Murrah No. 1	6.0	9.2
Murrah No. 6	8.8	10.4
Murrah No. 22	6.4	10.4
Murrah No. 24	7.2	9.8
Murrah No. 3099	6.4	9.4
Mean average	6.96	9.84

t-table

TREATMENT	MEAN	COMPUTED *t*
	day	
IVT	6.96	5.15 *
Y-C	9.84	

* Significant at one per cent probability level.

Semen with motility below 50 per cent could not be recommended for insemination, but in cases where semen with good motility would not be available, it would be worthwhile to use semen with motility of as low as 30 per cent from a prized or proven sire.

The motility of the IVT-diluted Murrah bull semen dropped below 50 per cent on the fifth day, whereas that of the yolk-citrate-extended semen was still maintained at about 50 per cent. The motility of the yolk-citrate-diluted sample in the refrigerator decreased to about 35 per cent on the ninth day, whereas that of the IVT-treated semen at room temperature, to 35 per cent on the seventh day of storage.

The livability of the samples treated with the IVT and the yolk-citrate extenders was longer with samples possessing higher initial motility (tables 6 and 7). At 50 per cent motility level, the length of storage for IVT-diluted bull semen did not increase much between the initial motility range of 60-69 and 70-79 per cent, and between 70-79 and 80-89 per cent; however, that of 80-89 per cent was one day longer than that of 60-69 per cent.

TABLE 6.—*Average number of days the sample with different initial motility range maintained 50 per cent motility*

INITIAL MOTILITY RANGE	BULL		MURRAH BULL	
	Treatments			
	IVT	Y-C	IVT	Y-C
per cent	*day*	*day*	*day*	*day*
60–69	3.33	3.55	3.2	4.3
70–79	3.88	5.50	4.6	5.6
80–89	4.33	6.00		

TABLE 7.—*Average number of days the samples with different initial motility range maintained 30 per cent motility*

INITIAL MOTILITY RANGE	BULL		MURRAH BULL	
	Treatments			
	IVT	Y-C	IVT	Y-C
per cent	*day*	*day*	*day*	*day*
60–69	6.50	9.00	6.80	9.30
70–79	6.78	9.25	7.07	9.93
80–89	7.67	11.93		

The yolk-citrate-extended bull semen with initial motility of 70-79 per cent stored longer by about two days than that with the initial range of 60-69 per cent. However, between 70-79 and 80-89 per cent, no marked difference was observed; but that of 80-89 per cent was two days longer than that of 60-69 per cent.

For both the IVT- and yolk-citrate-extended semen of Murrah bulls, the initial range of 70-79 per cent gave a difference of one day over the 60-69 per cent. No initial ranged of 80-89 per cent was observed from Murrah bulls.

At 30 per cent motility level, the difference observed between 60-69 and 70-79 per cent for IVT- and yolk-citrate-diluted semen

from both bulls and Murrah bulls was not marked. In bulls, however, initial motility of 80-89 per cent stored longer by about one day for IVT-extended semen and two days for yolk-citrate-extended semen, than that of 70-79 per cent. Initial motility of 80-89 per cent stored longer by one day for IVT and two days for yolk-citrate samples, which had a 60-69 per cent motility range. In all cases, yolk-citrate samples stored longer than the IVT samples within their respective initial motility ranges.

Semen, in two separate ejaculations, was collected from each bull within an interval of 10 minutes following a stimulation period of 20 minutes (table 8).

TABLE 8.—*Average volumes of two successive ejaculates within ten minutes*

EJACULATE	BULLS				AVERAGE
	Red Sindhi	Santa Gertrudis	Brown Swiss	Holstein	
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
1	3.50	4.40	5.70	2.70	4.08
2	4.38	5.25	5.83	4.12	4.90
Total	7.88	9.65	11.53	6.82	8.98
Average	3.94	4.82	5.76	3.41	4.49

EJACULATE	MURRAH BULLS					AVERAGE
	No. 1	No. 6	No. 22	No. 24	No. 3099	
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	
1	3.20	2.82	3.10	2.70	2.50	2.86
2	3.10	2.10	3.50	2.40	2.60	2.74
Total	6.30	4.92	6.60	5.10	5.10	5.60
Average	3.15	2.46	3.30	2.55	2.55	2.80

The results showed that in bulls, the second ejaculate was consistently greater in volume than the first. The average volume of the first ejaculate of the four bulls was 4.08 ml. and that of the second, 4.90 ml. or an average of 4.49 ml. for the two ejaculations. The Brown Swiss bull gave the highest average total volume of ejaculates among the bulls with 11.53 ml.; followed by the Sta. Gertrudis bull, 9.65 ml.; the Red Sindhi bull, 7.88 ml.; and the Holstein bull, 6.82 ml. All were mature bulls except the Holstein which was under two years of age.

In contrast to these results, the Murrah bulls did not show any marked difference between the first and second ejaculations and also between animals. The average volume per ejaculate of the five Murrah bulls was 2.80 ml. with an average total volume of 5.60 ml.

The volume of ejaculates from bulls was higher than that from Murrah bulls in both the first and second ejaculations. The total average volume of ejaculates from bulls was higher by 3.38 ml. than that from the Murrah bulls probably because of differences in size between the testicles of the two species. With respect to their body size, the testes of the Murrah bulls are smaller than those of the bulls.

Summary and Conclusion

1. The correlations between motility reading and vital staining for both bull and Murrah bull-extended semen in the two treatments were high.

2. Bull semen with sufficient motility for fertilization stored in IVT-ordinary room condition lasted three to four days, and that in the yolk-citrate-refrigerated condition, five days. The IVT-extended Murrah bull semen with good motility lasted for about four days at room temperature, whereas the yolk-citrate-extended semen, for five days in the refrigerator.

3. The IVT-extended semen of bulls with 30 per cent motility lasted only seven days and the refrigerated one, nine days; that of the Murrah bull semen, seven days and the refrigerated, ten days.

4. Semen with higher initial motility tended to store longer in both IVT and yolk-citrate diluents.

5. The average volume of ejaculates from bulls was greater than that from the Murrah bull, both in the first and second ejaculations.

6. Based on this preliminary study, IVT-extended bovine semen stored at room temperature can be used for insemination within four days storage. However, further study along this line must be based not mainly on its motility-reading aspect but also on actual inseminations, and more bulls within breed should be used for further studies.

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INTERCHANGED CHROMOSOMES IN RECIPROCAL TRANSLOCATION STOCKS OF RICE [1]

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WITH THREE TEXT FIGURES

Twenty-seven stocks of rice heterozygous for reciprocal translocations were cytologically studied to determine the number of chromosomes involved in each translocation. Nine stocks were found to each have an interchange of two pairs of chromosomes as shown by the ring or chain of four configuration at diakinesis and metaphase I. However, not all plants in these stocks showed such configurations. Some showed only bivalents at the same stage. The ring forming plant had an average pollen sterility of around 55 per cent and an ovule abortion of around 65 per cent, while plants with only bivalents had ovule and pollen sterilities ranging from 3.89 to 22.86 per cent and 8.08 to 18.98 per cent, respectively.

Eighteen stocks showed 12 bivalents at diakinesis. Seven of them had relatively low sterility. The other 11 stocks had plants with sterilities as high as those of the ring forming plants.

In general, high pollen abortion was accompanied by low seed set. In the highly sterile group (plants with more than 50 per cent sterility) ovule abortion was generally higher than pollen abortion.

Reciprocal translocations in plants, also termed as "chromosomal interchanges", have long received attention from cytologists and geneticists. This chromosomal aberration has been a useful tool in genetics. Anderson (1935) stated that the interchanges are useful in building up a knowledge of "such items as the location of given genes on the observed chromosome thread, the linkage relationships of conspicuous markers on the chromosomes; also to make available means of marking or of controlling desired portions of chromosomes."

One proposed use of reciprocal translocations is the production of homozygous inbred lines in a plant breeding program. This "Oenothera" method of plant breeding requires a ring involving the entire chromosome complement and can be applied to both cross- and self-pollinated crops (Burnham, 1946).

Chromosomal interchanges may arise spontaneously or as a result of irradiation or chemical treatments. The occurrence could be in the somatic tissue or in the germ cells during meiosis.

[1] Central Experiment Station Contribution No. 2046.

Translocations have been produced in rice by X-rays (Ramiah *et al.*, 1934; Oka *et al.*, 1953; Soriano, 1959). From X-rayed rice seeds, Ramiah *et al.* (1934) obtained a plant showing a ring of four chromosomes (O4) at diakinesis and metaphase I. This plant also showed 60 to 70 per cent pollen abortion. In a report by Oka *et al.* (1953), the sterility, ranging from 50 to 70 per cent, of several strains of X-rayed rice, was attributed to reciprocal translocation. An association of four chromosomes was also observed. Soriano (1959) isolated from a line of X-rayed rice, four plants with ring and chain configurations of chromosomes at diakinesis and metaphase I. The transmission of the reciprocal translocation and the standard normal condition to the X_2 was observed to be 1:1. He noted the seed set of the heterozygous plants and found that those bearing an interchange complex gave seed sets ranging from 16.25 to 50.72 per cent, which were much lower than those of the normal plants.

Twenty-seven stocks of rice which were heterozygous for reciprocal translocations were obtained from the United States. The interchanges in these stocks were induced by X-rays. Homozygous translocation stocks will be established from them which in turn will be used for both cytogenetic and plant breeding studies. The number of interchanges in these reciprocal translocation stocks is still to be determined.

The major purpose of this study was to find out the number of chromosomes which interchanged in the 27 reciprocal translocation stocks of rice. The degrees of pollen and ovule abortion were also studied and the plants suspected to be homozygous for the interchanges were isolated for further tests.

MATERIALS AND METHODS

Six plants from each of the 27 translocation stocks were grown in clay pots, simulating the lowland rice field conditions. Fertilizer was applied twice. The first application was made two weeks after the plants were transplanted and the second, before the booting stage.

Sporocytes were collected from each plant just before the flag leaf had fully emerged. They were fixed in Farmers' fluid (3 parts 95% alcohol: 1 part glacial acetic acid) for 24 hours and then preserved in 70 per cent alcohol. Cytological examinations were made,

using the acetocarmine smear technique. Chromosomal configurations at diakinesis and metaphase I stages were studied to determine the number of interchanges of each translocation stock.

The degree of pollen abortion of each stock was also determined with the iodine test. Shortly before anthesis, three to five florets were collected from each plant and preserved in 70 per cent ethyl alcohol. The anthers of each floret were placed in a drop of KI + I_2 solution on a slide and dissected to release the pollen grains. The drop was stirred before placing the cover slip in order to obtain a uniform distribution of the pollen grains. Countings were done on 10 different fields. Full and deeply stained pollen grains were considered fertile and the shrunken, unstained ones were classified as sterile. Three florets per plant were counted and the mean percentage of aborted pollen was used to represent the sterility of that particular plant.

The panicles that reached maturity were harvested and the seeds were counted. The undeveloped grains were classified as aborted ovules and their percentages were calculated.

The plants that showed configurations of 12 bivalents (12 II) and had low degrees of pollen and ovule abortion were isolated and the panicles were harvested separately. These plants will be grown in order to test their homozygosity to the interchanges.

RESULTS AND DISCUSSION

Out of the 27 stocks cytologically studied, only nine had plants that showed chromosome interchanges at diakinesis and early metaphase I. All of these nine stocks gave a configuration of a ring of four chromosomes and 10 bivalents (fig. 1), indicating that two chromosomes were interchanged in each. Most of the rings were open and only in very few instances were they arranged in the form-of-eight or zigzag fashion. In many of the cells, the four chromosomes were not completely joined; only a chain of four (fig. 2) could be seen. Two of these nine stocks, *Xt 828-9* and *BS-1-3A-9*, gave a very low frequency of the ring configuration. In some of the cells, 12 bivalents could even be observed. None of the nine stocks had all the six plants displaying chromosome catenation. Some of the plants showed 12 separate pairs of chromosomes (fig. 3). It was noted that the occurrence of the chain or ring configuration in the nine stocks was correlated with the degree of sterility of the plant. The plants exhibiting a ring or chain configuration had pol-

Fig. 1.—Camera lucida drawing of rice PMC showing a ring of four chromosomes and 10 bivalents. The ring of four chromosomes is the hollow circle-like figure surrounded by the bivalents.

Fig. 2.—Camera lucida drawing of rice PMC showing a chain of four chromosomes and 10 bivalents. The chain of four chromosomes is the elongated figure between the bivalents.

Fig. 3.—Camera lucida drawing of rice PMC showing twelve bivalents.

len and ovule abortions ranging from 50.78 to 60.72 per cent and from 50.74 to 70.48 per cent, respectively, while those giving 12 bivalents had a sterility from 8.08 to 18.89 per cent for the pollen and from 3.89 to 22.86 per cent for the ovule (table 1). On the average, the pollen abortion of ring forming plants was about 55 per cent, and the ovule abortion was around 65 per cent. Except for the low seed set, ring forming plants could not be visually detected from those with 12 bivalents.

TABLE 1.—*Average degree of sterility of stocks with a O 4 at diakinesis*

STOCK	CULTURE NUMBER	POLLEN ABORTION		OVULE ABORTION	
		Plants with O 4	Plants showing 12 II	Plants with O 4	Plants showing 12 II
		per cent	*per cent*	*per cent*	*per cent*
BS1–3A–9	1	55.83	8.08	78.48	18.80
BS3–3A–8	3	61.15	11.60	78.26	3.89
BS11–2–(15)	9	55.59	12.30	74.58	11.71
S14–8	13	59.54	16.77	50.74	22.86
S24–2	15	56.28	19.06	66.35	7.06
Xt828–9	16	60.72	10.75	56.26	12.68
Xt271–7	17	50.78	18.98	64.10	—
Xt889–10	23	56.70	—	73.40	22.30
Xt1239–9	27	53.86	11.09	63.95	16.69
Average		56.72	13.58	67.35	14.50

All the rest of the 27 stocks showed 12 pairs of chromosomes at diakinesis. Seven of these remaining stocks had a relatively low sterility. The degree of abortion of the pollen ranged from 11.20 to 28.40 per cent, and from 8.0 to 24.09 per cent for the ovule (table 2). The remaining 11 stocks showing 12 pairs of chromo-

TABLE 2.—*Stocks with low sterility and showing 12 II at diakinesis*

STOCK	CULTURE NUMBER	POLLEN ABORTION	OVULE ABORTION
		per cent	*per cent*
BS4–4–D–5	4	27.29	—
BS5–4–1–A	5	25.47	12.26
BS6–3–A–1	6	11.20	8.00
BS8–2A–2	7	18.97	21.63
BS10–2–(1)	8	12.84	24.09
Xt–902–1	25	17.86	17.78
Xt–957–9	26	28.40	13.23

somes at diakinesis had sterilities as high as those of the ring forming stocks. Their sterility ranged from 8.02 and 60.38 per cent and their pollen abortion, from 11.78 to 70.14 per cent (table 3).

Since the materials used in this study were obtained from heterozygous sources, plants with normal chromosome behavior, i.e. formation of 12 pairs of chromosomes at diakinesis and metaphase I, are expected to appear. These normal plants may consist of those

TABLE 3.—*Stocks with high sterility but showing 12 II at diakinesis*

STOCK	CULTURE NUMBER	POLLEN ABORTION		OVULE ABORTION	
		Group with low sterility	Group with high sterility	Group with low sterility	Group with high sterility
		(less than 50%)	(more than 50%)		
		per cent	*per cent*	*per cent*	*per cent*
BS2–3A–(2)	2	8.02	52.08	20.10	—
BS16–1–B(3)	10	10.28	55.81	14.60	63.40
BS18–2–1–25(2)	11	15.77	53.94	13.17	66.07
BS19–2–B–(2)	12	26.66	—	15.50	55.67
S17–6	14	15.22	—	24.39	57.64
Xt–872–9	18	11.96	52.11	19.49	57.64
Xt–873–8	19	19.08	—	20.14	51.24
Xt–876–6	20	24.14	60.30	18.63	—
Xt–881–8	21	23.80	58.56	13.53	59.86
Xt–882–10	22	10.84	51.02	30.12	70.14
Xt–899–7	24	12.58	51.84	11.78	—

without interchanges and those which are homozygous for reciprocal translocation or the so-called "modified" normals. In the nine stocks showing a ring of four, the plants which were observed to show 12 bivalents and which had low sterility, might have been homozygous for the standard normal condition or for the interchanges. The same explanation could be given for the failure of the seven stocks with low sterility to show a ring or chain configuration. The homozygosity of those plants could be tested by crossing them with standard normal plants and then examining the F_1 sporocytes cytologically. Plants homozygous for reciprocal translocation when crossed to standard normal ones would give F_1's which are all heterozygous for chromosomal interchange. Ring or chain of chromosomes would be observed by examining cytologically the F_1 pollen mother cell. On the other hand, 12 bivalents would be observed upon cytological examination of the F_1 sporocytes if those plants were homozygous for normal condition.

Not a single plant in the nine stocks gave a hundred per cent ring frequency. In some plants, a chain of four appeared more frequently than the ring configuration. Twelve bivalents even appeared in some cells of the ring forming plants of two stocks. Burnham (1956) considered relative arm length of the interchanged chromosomes as one factor controlling the frequency of ring configurations in translocated plants. He said that the configuration at diakinesis and metaphase I will be rings when the interchanged pieces are long.

chains and rarely rings when one long piece has exchanged with a very short one, and chains or separate pairs and no ring at all if both pieces are short. It is possible that the exchanged chromosome segments of the nine stocks, especially *Xt 828-9* and *BS 1-3A-9* were those of a long piece of one chromosome and a very short arm of the other, hence, the observed configurations. Oka *et al.* (1953) also mentioned chiasma frequency as a probable cause of difference in the frequency and shape of quadrivalent chromosomes. This is, however, related to Burnham's hypothesis since they assumed that the chiasma frequency is directly proportional to the chromosome length.

On the average, the degree of sterility of ring forming plants, both pollen and ovule, was over 50 per cent, indicating the high frequency of adjacent segregation of chromosomes at anaphase I. This agrees with the findings of Soriano (1959). The occasional zigzag configurations suggested further the rather low frequency of alternate segregation in these stocks.

The failure to observe either a ring or a chain configuration among the highly sterile plants of the eleven stocks may be due to the interchange of very short portions of two chromosomes. It might be also that the translocation is of the intercalary type. Since in this kind of interchange, the internal segment of one chromosome is inserted between two segments of another, the ends of the chromosomes involved, obviously, would not fuse. Another possible reason for the normal cytological behavior of the chromosomes in the eleven stocks is the fact that some chromosomes of rice are rather short, from 0.7 to 2.8 microns, so that interchanges among them would not be observable. In species with very short chromosomes, translocations may be present but not indicated by any observable association oof chromosomes (Burnham, 1956). The shortness of the chromosomes would prevent much crossing-over, so the four-armed figure at pachytene would seldom open into a ring or chain.

However, the degree of sterility may indicate the number of chromosomes involved in the interchanges. Since the sterility determination of these eleven stocks did not vary much from the ones found with ring or chain configurations, it is possible that the reciprocal translocation in each stock involves two chromosomes.

In general, high pollen sterility was accompanied by low seed set. However, ovule abortion was generally higher than pollen abortion in the highly sterile group (sterility of more than 50 per cent).

This difference might be attributed to the method employed in determining ovule abortion as well as to environmental factors. The frequent occurrence of bad weather during the growing period of the experimental plants could have decreased the percentage of pollination, thus increasing the number of undeveloped ovules. The percentage of pollen abortion could therefore be considered a more reliable measure of the degree of sterility of individual plants.

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EFFECTS OF X-RAY ON THE BULBS AND SEEDS OF ONION (ALLIUM CEPA L.)[1]

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WITH THREE TEXT FIGURES

Results obtained from subjecting onion seeds to various levels of X-rays showed that germination was relatively unaffected. X-ray levels between 50r and 100r resulted in stimulation of growth in terms of height and fresh weight, the optimum dosage being at 75r. No significant difference in number of leaves was obtained from among the treated lots. An atypical plant was isolated from the lot subjected to 75r. This plant exhibited fasciation and proliferation of the leaves and upon maturity it produced four distinct bulbs.

The percentage of germination of the bulbs indicated an inverse relation to X-ray dosage. The sprouting period was relatively unaffected by the dosage treatments. No sign of flowering was observed.

The effects of X-ray and other ionizing radiations on plants especially on seeds have been studied for a long time. However, due to insufficient information on their proper use, different results have been obtained. Generally, the effects of these radiations on plants have been largely deleterious, even lethal in cases where heavy doses were applied, although different species of plants vary greatly in their radio-sensitivity.

Light dosages of ionizing radiations applied to seeds have been reported to stimulate plant development, resulting in earlier anthesis and greater yields, while heavy doses were deleterious and even lethal (Shull and Mitchell, 1933; Johnson, 1948; Shebeski and Lawrence, 1954; Mackey, 1954). Koernicke (1904, 1915) observed some stimulation of plant growth following light doses of X-rays, but in general the effect was deleterious. Sax (1955), in a critical study of x-irradiation effects on dry seeds of various crop plants, noted some stimulation of growth but found no significant effect on yield. Johnson (1936) observed that low X-ray doses stimulated the growth of *Mesembryanthemum* and *Sedum* resulting in taller, more-branched plants with a greater average dry weight than the controls.

[1] Central Experiment Station Contribution No. 2047.

Little work has been done on the effects of radiations on asexually reproductive parts of the plants. Sax (1955) reported that X-rayed *Gladiolus* bulbs bloomed significantly earlier than the controls when exposed to 4,000r (roentgen) and suggested that higher dosages might be more effective. He also stated that X-irradiated *Gladiolus* bulbs at 2,500r and 5,000r resulted in earlier germination. However, dosage levels beyond 5,000r had a significant delaying effect on germination. Spencer (1955) reported that the exposure to X-rays at 5,200r of bulbs and corms of 16 varieties from 12 genera of cultivated, monocotyledonous plants resulted in significantly earlier flowering.

Although one may find papers suggesting the stimulatory effects of X-rays at low doses, proof to such action has been wanting. Other investigators found that ionizing radiation did not produce any stimulatory or beneficial effect on plant growth (Johnson, 1931; Dittrich *et al.*, 1949; Alexander, 1950). More recent findings denied the stimulative action of X-ray, believing, on the contrary, that X-rays are always more or less destructive and that they tend to retard growth.

Most of the reports of increased yields or growth stimulation following x-irradiation of seeds or bulbs are doubtful due to inadequate data to support them. However, there is some critical evidence of plant growth stimulation in a few cases. Shull and Mitchell (1933) believed that stimulative effects may be consistently obtained if appropriate conditions are employed. He suggested that each kind of plant probably has its own specific optimum dosage. Such dosage specificity is determined by experiment for each species and varietal strain.

This investigation was conducted from April, 1959 to January, 1960, in the Department of Agricultural Botany, U.P. College of Agriculture to (1) observe the immediate effects of X-ray on seeds and bulbs of onions and (2) determine whether flowering could be induced by subjecting bulbs and seeds to varying dosages of X-rays.

MATERIALS AND METHODS

Experiment on seeds. Seeds of the Excel variety were subjected to X-rays at dosage levels of 25r, 50r, 75r, 100r, 150r, 200r, and 500r. A subsequent control was maintained. X-rays were applied without filter by a General Electric Maximar 100, operated at 225r

per minute, 100 kv (kilo voltage), 50 ma (milliampere) and at a target distance of 15 centimeters.

One hundred seeds for each dosage lot were irradiated and immediately sown with its control, at a distance of one centimeter from each other on separate seed boxes containing a previously heat-sterilized medium. The medium was composed of garden soil, fine sand, and compost of equal proportion. The number of days from sowing to germination and the percentage of germination were recorded. About a month after sowing, 20 seedlings from each treatment were taken at random and transplanted in pots containing the same medium used in germination. One seedling was planted to a pot. Two weeks after transplanting, weekly height measurements were obtained. The height and the number of leaves of the plants were taken four months after sowing. Completely randomized design was employed in the analysis of the data.

Experiment on bulbs. Ten bulbs of each variety (Excel and Granex) were exposed to 500r, 1,250r, 2,500r, 3,750r, 5,000r of X-rays. The same X-ray apparatus used in the seed treatment was employed. The irradiated bulbs were immediately planted together with the control, in pots with the same soil medium used in the experiment with seeds. One bulb was planted to a pot. The number of sprouted bulbs and the days to germination were observed.

The standard cultural practices recommended for cultivation of the varieties under study were followed.

RESULTS AND DISCUSSION

Experiment on seeds. Germination among the treatments was observed a week after the irradiated seeds were sown. Table 1 shows the percentage of germination of the control and the treated lots based on 100 seeds. Although the data were inadequate for statistical analysis, it could be seen that the percentage of germination was relatively unaffected.

TABLE 1.—*Percentage of germination of onion seeds (Excel var.) following treatment with various X-ray dosages*

DOSES	PERCENTAGE OF GERMINATION	DOSES	PERCENTAGE OF GERMINATION
0	63	100r	62
25r	66	150r	67
50r	70	200r	64
75r	65	500r	68

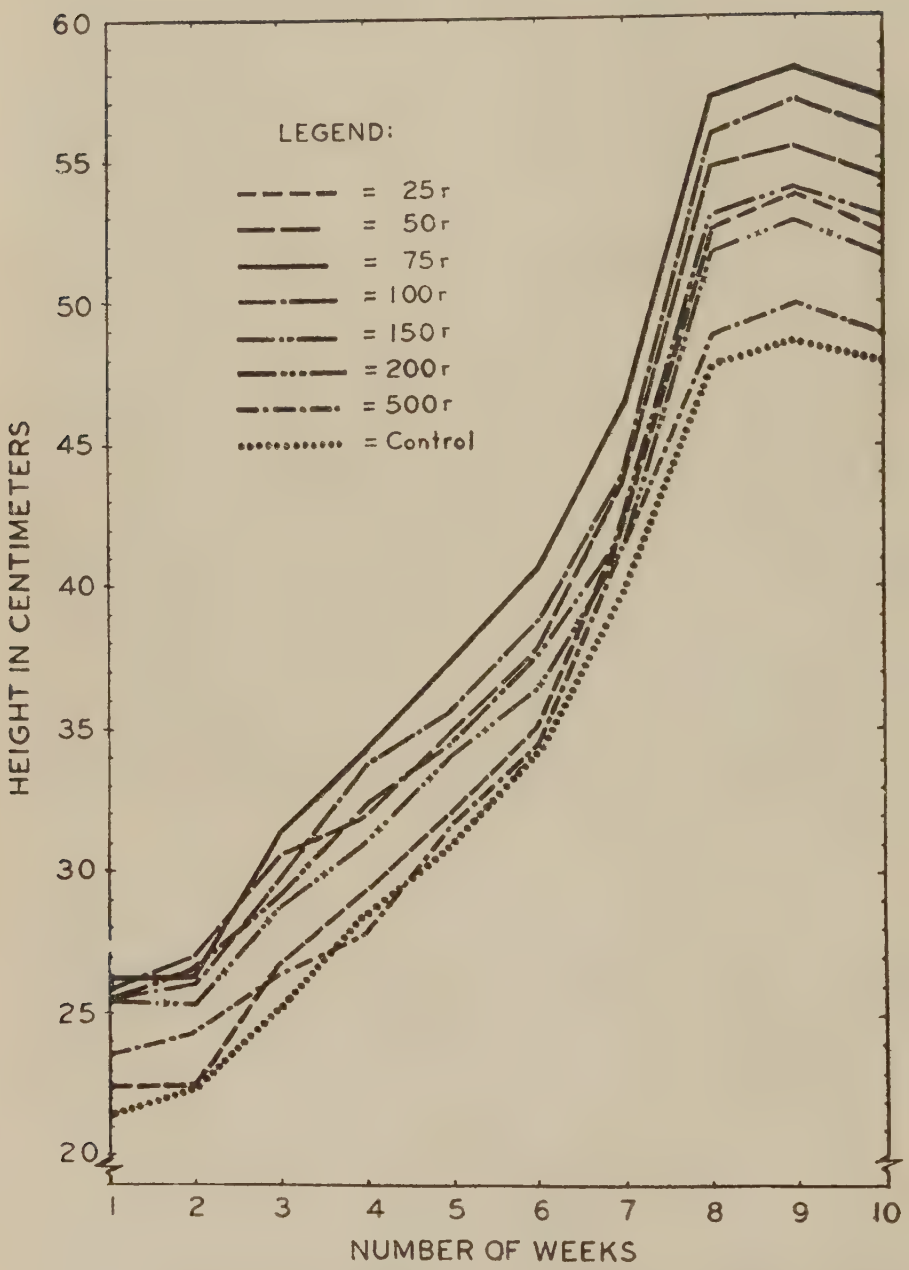

Fig. 1.—Rate of growth of the Excel variety grown from seeds treated at different levels of x-ray.

This result could be attributed to the rather low and close ranges of dosages employed. It is also possible that ants had eaten some of the endosperm of the seeds since they were observed in the seed boxes even after the seedlings sprouted.

Two weeks after transplanting, and until the plants reached maturity, weekly height measurements were taken. Figure 2 shows

Fig. 2.—Growth comparison of the Excel variety grown from seeds treated at different levels of x-ray.

the rate of growth of the plants. Regardless of treatment, the plants exhibited relatively the same rate of growth.

Table 2 shows the average height of four-month old onion plants. It could be noted that as the dosage of X-rays increases up to 75r, there is a corresponding increase in height. However, the effect of the treatments ranging from 100r to 500r seemed to be inversely related to the height of the plants. Upon comparing the treated lots with the control, plants at 50r, 75r, and 100r were found to be taller than those of the control. Johnson (1936) found the same effect in addition to the observed increase in weight in her radiation experiments with *Mesembryanthemum* and *Sedum.*

To further show the response of the Excel variety when its seeds were exposed to varying dosages of X-ray, a representative plant from each treatment lot was taken and photographed (fig. 2). From the figure, a similar trend as in table 2 can be observed.

Table 3 shows the average number of leaves of four-month old onion plants. Statistical analysis of the data indicated that there were no significant differences between the treated lots and the control.

TABLE 2.—*Height of the plants grown from X-rayed seeds at the age of four months (centimeter)*

REPS	DOSAGE							
	0	25r	50r	75r	100r	150r	200r	500r
1	56.5	55.0	77.5	61.5	55.0	59.5	45.0	50.0
2	53.0	67.5	56.0	53.0	59.5	58.0	59.5	50.5
3	45.5	58.0	58.0	55.0	58.0	55.5	62.0	54.0
4	50.0	61.5	51.5	58.5	59.0	50.0	50.5	60.0
5	52.0	48.5	52.0	68.5	58.5	59.5	61.5	49.5
6	38.5	60.5	48.0	53.0	62.0	52.0	59.0	50.0
7	59.0	43.0	56.0	65.5	57.5	59.5	44.0	39.5
8	50.5	52.5	60.5	65.0	59.5	56.0	63.5	56.0
9	43.0	58.0	57.0	63.5	56.5	57.5	63.0	49.5
10	57.0	57.0	66.5	74.0	69.0	61.0	54.5	65.0
11	41.5	42.0	57.5	40.0	39.5	36.5	41.5	47.0
12	55.0	50.5	49.0	55.0	40.0	47.0	54.0	53.5
13	52.5	58.0	52.0	52.5	48.5	48.0	46.0	50.5
14	45.0	54.5	53.5	54.0	57.0	56.5	51.5	53.0
15	40.0	59.5	48.0	50.5	57.0	56.0	42.0	47.5
16	47.5	59.0	52.0	57.0	58.5	49.0	40.0	51.0
17	46.0	49.0	47.5	55.0	59.0	54.0	43.0	44.5
18	54.5	55.5	55.0	63.5	55.5	55.5	61.5	42.0
19	41.0	46.0	49.0	58.0	65.0	54.0	54.5	46.0
20	44.5	42.0	60.5	59.5	66.0	55.0	59.0	36.5
Average	48.63	53.88	55.35	58.13	57.03	54.00	52.78	49.78

HSD .05 = 6.69 HSD .01 = 7.78

An atypical plant was isolated from the lot treated at 75r (fig. 3). This plant exhibited normal growth from germination to its third month. However, upon maturity, there was a proliferation and fasciation of numerous small leaves. When harvested, four distinct bulbs were obtained.

When the fresh weight of the onion plants was taken from each dosage lot, an effect similar to that in height was observed (table 4). A significant difference was found when X-ray treated lots were compared to the control. Among the treated lots, plants at 75r were heavier than the control

Again, it can be deduced that X-ray treatments of onion seeds produced stimulatory effects at low dosages (the optimum being 75r) resulting in heavier and taller plants.

Although several workers (Johnson, 1931; Dittrich *et al.*, 1949; Alexander, 1950) denied the stimulative action of X-ray and dis-

TABLE 3.—*Number of leaves of the plants grown from X-rayed seeds at the age of four months* [a]

REPS	DOSAGE							
	0	25r	50r	75r	100r	150r	200r	500r
1	7	8	6	8	7	7	5	7
2	8	5	5	8	7	6	7	6
3	6	8	5	7	7	6	8	6
4	5	7	8	8	6	8	9	5
5	7	5	6	8	7	6	5	6
6	5	7	6	6	4	7	8	5
7	6	5	7	7	6	5	6	6
8	7	5	9	8	6	7	7	9
9	7	8	8	8	8	7	6	6
10	6	8	9	9	10	6	7	7
11	7	6	8	4	4	6	7	7
12	7	6	8	7	5	6	6	7
13	6	7	6	6	6	6	6	6
14	6	6	5	6	7	5	6	7
15	4	7	6	6	7	8	6	6
16	5	6	7	6	6	5	6	7
17	7	4	6	7	8	6	6	5
18	7	6	6	6	8	5	7	6
19	8	5	7	6	8	7	6	7
20	6	7	9	8	8	7	8	5
Average	6.35	6.30	6.85	6.95	6.75	6.30	6.60	6.30

[a] No significant difference.

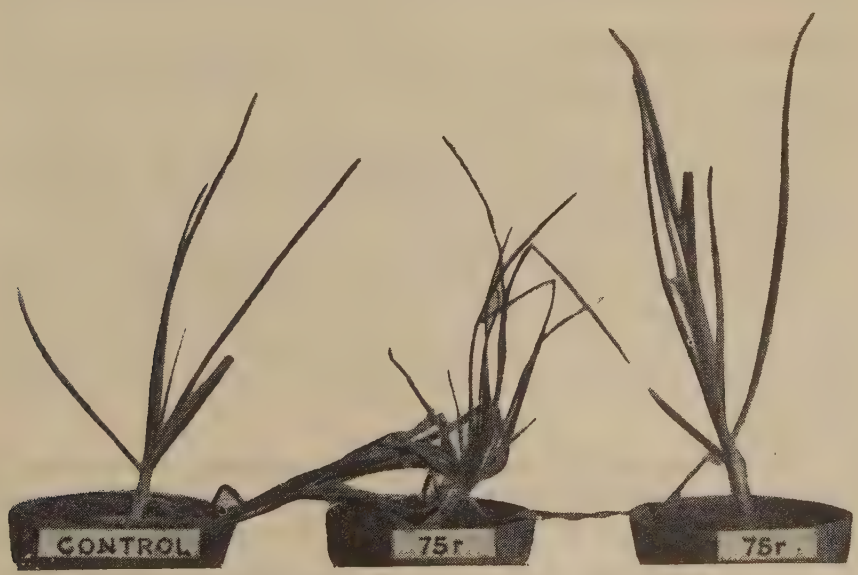

Fig. 3.—(A) Control; (B) an atypical plant isolated from the lot treated at 75r; and (C) phenotypically normal plant from lot exposed at 75r.

TABLE 4.—*Weight of plants at maturity (grams)*

REPS	DOSAGE							
	0	25r	50r	75r	100r	150r	200r	500r
1	59.4	81.9	113.0	123.5	80.2	54.2	43.5	50.3
2	48.5	107.1	50.0	67.2	58.6	71.9	103.7	64.6
3	66.2	70.4	75.3	106.1	55.9	74.8	109.3	55.8
4	53.6	120.7	52.1	91.3	104.1	74.2	70.7	88.7
5	68.3	38.2	67.8	103.0	57.6	54.9	35.0	37.5
6	40.7	67.4	34.9	107.6	58.3	54.4	60.3	71.5
7	44.8	56.4	79.5	80.0	59.0	43.9	41.2	41.5
8	55.1	39.3	144.2	95.7	93.7	68.6	78.0	56.9
9	54.3	67.7	50.4	71.8	57.8	21.2	59.3	79.8
10	59.7	81.8	88.4	47.2	10.7	40.5	30.0	20.6
11	39.7	44.8	80.0	55.4	21.6	43.2	59.8	68.6
12	42.3	45.9	48.1	43.8	30.3	34.4	25.2	25.1
13	25.0	18.4	29.8	70.9	42.1	45.9	51.7	28.8
14	32.3	41.2	17.3	49.8	63.2	40.6	22.2	52.4
15	13.6	36.5	37.8	54.4	65.5	41.8	26.4	10.8
16	17.2	48.6	43.5	58.4	54.7	42.3	42.1	
17	40.4	45.2	41.2	58.6	58.3		60.6	
18	35.8	28.1	34.0	58.7	102.1		47.5	
19	77.4	47.0	43.5		98.8		71.8	
20	35.8	49.7	110.1					
Average	45.51	56.82	62.05	74.63	61.71	51.68	54.65	50.19

HSD .05 = 24.11

counted its beneficial effects, the results of this experiment proved otherwise. These might have been due to the use of very light doses.

Studies on induction of flowering from irradiated seeds were not continued since the bulbs produced from such treated seeds rotted, and therefore could not be replanted.

Experiment on bulbs. The Excel and Granex varieties did not differ much in the length of time of sprouting when their bulbs were previously subjected to varying X-ray dosages. However, within each variety, it was observed that sprouting was delayed among the treated lots.

Table 5 shows that as the dossage increased, the number of bulbs that sprouted decreased. Kehr [2] observed that when *Gladiolus* bulbs were subjected to 10,000r, germination was significantly de-

[2] As reported by Sax (1955) from a personal communication with Kehr.

TABLE 5.—*Number of germinated bulbs exposed to different levels of X-rays*

DOSAGE	GRANEX	EXCEL	DOSAGE	GRANEX	EXCEL
0	9	10	2500r	5	5
500r	8	8	3750r	4	5
1250r	6	7	5000r	5	3

layed, and at 20,000r the germination was only one third of the control. The control lots gave 100 per cent germination for Excel and 90 per cent for Granex.

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EFFECT OF SEED TREATMENT WITH MALEIC HYDRAZIDE ON GERMINATION AND SEEDLING GROWTH OF CORN [1]

IBARRA S. SANTOS [2]

Seed treatment with maleic hydrazide, including soaking in maleic hydrazide solution or storage with maleic hydrazide in powder form, resulted in a delay of at most one day in emergence of corn seedlings. There was an indication that seeds stored with maleic hydrazide lost their viability faster than the untreated seeds. Seedlings grown from seeds soaked in maleic hydrazide solution grew very slowly and died sooner or later. Seedlings grown from seeds stored with maleic hydrazide for 12 or more days also died. There is still hope that seedlings from seeds stored with maleic hydrazide for eight days or less may live to reach maturity.

In the production of double-crossed seeds, the advantage of planting the two parental single crosses at the same time has been recognized. This ordinarily could not be done if the two parental single crosses differ in relative maturity, which is often the case. In such instances, a method of hastening the flowering of the later-maturing single cross or of delaying that of the earlier-maturing single cross has to be employed if the two single crosses differing in maturity are to be planted at the same time. Santos, de Mesa, and Landicho (1959) obtained, on the average, as much as 6.7 days acceleration in days from planting to silking in the 1956-57 dry season in the Philippines. In this and other seasons, however, the hastening effect on days to silking was not enough to cover the difference between the days to silking of the earlier parent and the days to pollen-shedding of the later parent in spite of the additional advantage accruing from the fact that pollen-shedding was always ahead of silking in all the single crosses studied.

The growth inhibiting effect of maleic hydrazide (MH) is well known. Schoene and Hoffman (1949) showed that MH can cause cessation of terminal growth without permanent injury to the plant. Other workers, particularly Naylor and Davis (1950), found that

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[2] The writer is grateful to Messrs. Benito L. Lit and Rosendo K. Palis for their assistance in the conduct of the six experiments reported in this paper, and to Miss Azucena L. Carpena for her help in the summarization of data.

loss of apical dominance is a characteristic response of a wide range of plants to this chemical. Most probably, this occurs as a result of inhibition of cell division in the apical meristem since interference with mitosis is one of the best established effects of MH on plants (Greulach and Atchison, 1950). Darlington and McLeish (1951) found that MH inhibits mitosis and causes chromosome breakage. Greulach (1951) and Zukel (1952) also noted that MH inhibits cell division rather than cell elongation.

MH is readily absorbed by roots and leaves and is generally translocated in both upward and downward directions. However, James and Chadwick (1959) observed in *Taxus media* Brown that MH was translocated only basipatally (downward) and that it accumulated in necrotic tissues, younger leaves and terminal buds on treated branches, phloem tissue, and throughout the root system, especially the root tip. They concluded that MH diffused more readily through dead tissues than through live tissues. Brown and Hitz (1957), working on strawberry runners, noticed the tendency of MH to withdraw from the less physiologically active to the more physiologically active tissues such as the actively growing points "for some time until the position of the MH becomes fixed."

The writer knows of no investigator who has conducted experiments on maleic hydrazide treatment of seeds. The experiments reported in this paper were conducted to obtain basic information on the effect of MH seed treatment on germination and early seedling growth of corn and to consider the possibility of either delaying germination of the corn seeds or retarding the maturity of the corn plant so as to lengthen the period between planting and flowering. These experiments were performed in the Plant Breeding Division, Department of Agronomy, U.P. College of Agriculture, from November, 1955 to April, 1957.

MATERIALS AND METHODS

Experiment 1

Maleic hydrazide (40 per cent pure) used in all the experiments was obtained from U.S. Rubber, Naugatuck, Connecticut through the Vegetable Section, Division of Horticulture, Department of Agronomy. In this experiment, the following concentrations of maleic hydrazide aqueous solution were made: 10, 5, 2, 1.5, 1.0, and 0.5 per cent. These solutions were made by dissolving in 100 milliliters

of water 25.00, 12.50, 5.00, 3.75, 2.50 and 1.25 grams, respectively, of maleic hydrazide powder.

Seeds of College Yellow Flint, a high-yielding open-pollinated variety, were used in this experiment. The corn seeds were pre-soaked in water for 24 hours. On November 28, 1955, the pre-soaked seeds were placed in the respective solutions and were allowed to stay there for about a minute. Without being rinsed, 25 seeds from each treatment were planted in the soil on the same day.

Four days after planting (December 2, 1955), the seedlings in the control (pre-soaked but not treated with MH) and in the 0.5 per cent treatment started to show up while those in the other treatments did so only the following day. Seedlings in the 1.0 per cent treatment seemed slow in coming up.

The percentage of germination in the control lot was 64; those in the MH treatments varied from 50 to 60 per cent.

The "treatment" seedlings grew very slowly and their leaves hardly opened. All, except two, were dead 36 days after planting (January 3). One of these surviving "treatment" seedlings was vigorous; the other was weak. "Control" plants all grew normally.

Experiment 2

Seeds of (A102 x A106) (A110 x A115) were pre-soaked in tap water for 26 hours. They were later placed in maleic hydrazide solutions of different concentrations (see experiment 1) and allowed to stay there for two minutes with vigorous stirring. Twenty-five seeds for each treatment were sown immediately after treatment on January 18, 1956.

Table 1 summarizes the percentage of germination and the final percentage of survival. In addition to the control, seeds soaked for 26, 52, and 102 hours were also planted although on slightly later dates. The percentage of germination in the 0.50 per cent treatment (72) was obviously low. The percentages of survival in the 0.20 and 0.50 per cent treatments were very low. Both suffered a mortality of 68 percent.

Table 2 shows the height frequency distribution taken on January 25, about seven days after planting, when differences in rate of growth were already very obvious. Plants in the 0.20 and 0.50 per cent MH treatments were "stunted". After germination, most

of the plants in these treatments hardly grew. The leaves turned pale (or white) and most of the plants ultimately died (table 1).

TABLE 1.—*Percentage of germination and final percentage of survival taken on February 20, 1956*

TREATMENT	DATE PLANTED (1956)	PERCENTAGE OF GERMINATION		SURVIVAL
		Jan. 21	Jan. 25	
Control (unsoaked)	January 18	8	100	100
Control (soaked 36 hours)	January 18	80	96	96
0.01% MH	January 18	64	92	84
0.05% MH	January 18	92	100	84
0.10% MH	January 18	80	100	88
0.20% MH	January 18	48	92	24
0.50% MH	January 18	20	72	4
Soaked 52 hours	January 19	8	96	96
Soaked 102 hours *	January 21	—	47	47

* 15 seeds only.

TABLE 2.—*Height frequency distribution on January 25, 1956*

	TREATMENTS						
	Control (unsoaked)	Control (soaked 26 hrs.)	0.01% MH	0.05% MH	0.10% MH	0.20% MH	0.50% MH
No. germinated	25	24	23	25	25	23	18
Height classes							
0–2.0	—	—	—	1	1	10	12
2.1–4.0	—	—	—	—	—	1	4
4.1–6.0	—	—	2	—	1	3	2
6.1–8.0	—	—	2	1	2	5	—
8.1–10.0	1	—	2	3	1	—	—
10.1–12.0	5	1	5	3	3	—	—
12.1–14.0	10	2	3	3	4	3	—
14.1–16.0	8	5	2	3	2	1	—
16.1–118.0	1	16	7	11	11	—	—
Mean height (cm.)	13.2	16.0	12.4	13.6	13.2	5.1	1.9

Experiment 3

Seeds of (A102 x A107) (A111 x A113) originally treated with DDT [2] were washed with water and dried. Unwashed seeds were used for the control treatment. Washed and unwashed seeds were stored with MH in capped bottles measuring six centimeters both in height and in diameter. Two hundred seeds were placed in each

[2] Dichloro-diphenyl-trichloroethane, 50 per cent pure wettable powder.

bottle. In treatments involving MH, about 30 grams of 40 per cent pure maleic hydrazide were placed with the seeds in the bottle. Once a week, the bottles were shaken.

Twenty-five seeds from each treatment were sown on March 22, 1956 and on September 15, 1956.

Table 3 gives the percentages of germination and of expected survival as of April 5, 1956 from each treatment in the sowing done on March 22, 1956, about two months after storage. The plants from seeds treated with maleic hydrazide (MH) had very much lower percentages of survival than the control.

TABLE 3.—*Percentage of germination and of expected survival from sowings on March 22, 1956*

	CONTROL (DDT)	MH	MH + DDT
Date seeds stored in bottles	Jan. 25, '56	Jan. 18, '56	Jan. 17, '56
		per cent	
Germination, March 27, 1956	96	96	100
Expected survival, April 5, 1956	96	24	12

Table 4 gives the percentages of germination and of survival from sowings done on September 15, 1956. There were two replications. As an average of the two replications, control gave 88 per cent, MH + DDT, 60 per cent germination. All the seedlings in the maleic hydrazide treatments died prematurely.

TABLE 4.—*Percentage of germination and of survival from sowings on September 15, 1956*

TREATMENT	REPLICATION	PERCENTAGE OF GERMINATION		SURVIVAL
		Sept. 20	Sept. 25	
Control (DDT)	I	36	80	80
	II	72	96	96
MH	I	44	60	0
	II	56	80	0
MH + DDT	I	44	72	0
	II	40	52	0

Experiment 4

Seeds of Cuban Yellow Flint single cross A112 x A113 were used. For the (0) or control treatment, the seeds previously treated with DDT were washed with water and dried. For the DDT treatment, seeds previously treated with DDT were placed in the bottles. For the MH treatment, the washed seeds were placed in bottles with 10 grams of 40 per cent pure maleic hydrazide powder. For the MH + DDT treatment, unwashed seeds were placed in bottles with 10 grams of maleic hydradize powder. Eighty seeds were placed in each bottle. There were two bottles for each treatment, one designated as Replication I and the other as Replication II. The bottles measured nine centimeters in height and 4.5 centimeters in diameter.

The seeds were placed in the bottles on September 28, 1956. Three weeks later, and at two weeks interval after this until the 13th week, 10 seeds from each replication were sown. The percentage of germination was observed three or four days after sowing and almost every day after this until it became obvious that all the viable seeds had germinated. About eight days after planting, the height of the plants was measured. Height was taken as the distance from the ground level to the top of the extended longest leaf on the plant.

The results are summarized in table 5. Three weeks of storage was enough to demonstrate the effect of maleic hydrazide on the growth of the corn plants. In subsequent plantings, the effect of maleic hydrazide on the growth of the corn plants became more evident. However, a coating of DDT powder served to decrease the detrimental effect of maleic hydrazide; the height of plants in the MH + DDT treatment was always (except in the last planting) greater than that of the plants in the MH treatment.

The effect of maleic hydrazide on germination percentages was not very clear. There was an indication, though, that seeds stored with maleic hydrazide powder (with or without DDT) lost their viability faster than the seeds in the control (0) and the DDT treatments.

The seedlings in the MH and MH + DDT treatments seemed to have a slightly slower rate of emergence than those in the control and DDT treatments. They were pale and weak, and they died prematurely.

TABLE 5.—*Percentage of germination of corn seeds stored for 3 to 13 weeks in bottles with and without maleic hydrazide and the mean height of plants eight days after sowing*

WEEKS AFTER STORAGE	DATE OF PLANTING	TREATMENTS [a]	PERCENTAGE OF GERMINATION AND DAYS AFTER PLANTING			MEAN HEIGHT (cm.)
			3	4	8	
3	Oct. 19, 1956	0	65	90	95	16.0
		DDT	50	75	85	14.6
		MH + DDT	45	95	95	8.6
		MH	45	80	90	3.0
5	Nov. 2, 1956	0	75	90	95	18.4
		DDT	75	90	90	16.9
		MH + DDT	50	65	70	9.6
		MH	35	80	80	1.8
7	Nov. 15, 1956	0	—	75	85	22.3
		DDT	—	75	85	25.2
		MH + DDT	—	55	60	9.2
		MH	—	70	80	1.6
9	Dec. 3, 1956	0	—	45	55	9.2
		DDT	—	35	45	14.2
		MH + DDT	—	10	30	3.4
		MH	—	35	65	1.7
11	Dec. 17, 1956	0	—	40	55	18.1
		DDT	—	20	35	20.3
		MH + DDT	—	20	30	2.1
		MH	—	15	40	1.7
13	Dec. 31, 1956	0	—	15	25	7.7
		DDT	—	10	15	13.1
		MH + DDT	—	0	0	—
		MH	—	0	10	1.4

[a] 0 = no treatment, or control.
DDT = seeds treated with dichloro-diphenyl-trichloroethane.
MH + DDT = seeds previously treated with DDT.
MH = seeds washed of DDT and then treated with maleic hydrazide.

Experiment 5

The materials and methods used were exactly the same as those in experiment 4, except for the following: (a) there were 125 seeds of A112 x A113 for each bottle, instead of 80, (b) the first planting was done nine days after storage, instead of after three weeks, and (c) the seeds were placed in the bottle on November 17, 1956.

The results are summarized in table 6. Obviously, the A112 x A113 seeds used in this experiment had a lower percentage of germination than those used in experiment 4.

TABLE 6.—*Percentage of germination of corn seeds stored for 9 to 37 days in bottles with and without maleic hydrazide, and the mean height about 10 days after planting*

DAYS AFTER STORAGE	DATE OF PLANTING	TREATMENTS	PERCENTAGE OF GERMINATION AND DAYS AFTER PLANTING		MEAN HEIGHT (cm.)
			5	9	
9	Nov. 26, '56	0	25	45	16.5
		DDT	20	30	16.4
		MH = DDT	30	40	6.2
		MH	35	45	2.7
16	Dec. 3, '56	0	20	20	14.5
		DDT	15	15	13.1
		MH + DDT	15	15	3.6
		MH	5	5	1.3
23	Dec. 10, '56	0	35	40	20.4
		DDT	45	55	20.0
		MH + DDT	30	45	4.5
		MH	15	40	1.2
30	Dec. 17, '56	0	15	20	18.9
		DDT	15	15	15.8
		MH + DDT	5	5	6.5
		MH	5	15	1.6
37	Dec. 24, '57	0	35	35	19.5
		DDT	5	5	10.0
		MH + DDT	10	10	10.0
		MH	10	15	3.6

As early as nine days after storage, the effect of maleic hydrazide on the growth of the plants was already obvious. DDT, in somewhat reducing the harmful effect of maleic hydrazide again showed the same effect.

No clear effect of maleic hydrazide on the percentage of germination was indicated.

Almost all the seedlings in the MH and MH + DDT treatment died prematurely.

Experiment 6

The materials and methods used were exactly the same as those in experiment 5, except for the following: (a) seeds of A102 x A106, instead of A112 x A113, were used and (b) there were 150 seeds per bottle, instead of 125. The seeds were placed in the bottles on March 19, 1957.

The results are summarized in table 7. After eight days of storage, the effect of maleic hydrazide on the height of the corn plants was already discernible. After 14 days of storage, the effect became drastic. The seedlings in the MH + DDT and MH treat-

TABLE 7.—*Percentage of germination of corn seeds stored for 2 to 30 days in bottles with and without maleic hydrazide and the mean height 10 days after planting*

DAYS AFTER STORAGE	DATE OF PLANTING	TREATMENTS [a]	PERCENTAGE OF GERMINATION AND DAYS AFTER PLANTING		MEAN HEIGHT (cm.)
			3	11	
2	Mar. 21, '57	0	100	100	30.6
		DDT	95	95	33.5
		MH + DDT	90	100	30.1
		MH	85	100	29.6
4	Mar. 23, '57	0	85	100	31.9
		DDT	95	100	33.6
		MH + DDT	95	100	32.1
		MH	90	100	29.2
6	Mar. 25, '57	0	100	100	37.5
		DDT	100	100	36.7
		MH + DDT	100	100	35.5
		MH	95	95	38.8
8	Mar. 27, '57	0	85	90	34.1
		DDT	100	100	32.9
		MH + DDT	100	100	27.3
		MH	95	100	28.3
10	Mar. 29, '57	0	100	100	31.0
		DDT	100	100	33.4
		MH + DDT	100	100	30.1
		MH	100	100	24.7
12	Mar. 31, '57	0	100	100	37.0
		DDT	100	100	37.2
		MH + DDT	95	95	26.1
		MH	100	100	25.8

DAYS AFTER STORAGE	DATE OF PLANTING	TREATMENTS	PERCENTAGE OF GERMINATION AND DAYS AFTER PLANTING		MEAN HEIGHT (cm.)
			3	11	
14	Apr. 2, '57	0	85	95	31.5
		DDT	70	100	31.3
		MH + DDT	60	100	9.4
		MH	70	95	11.3
16	Apr. 4, '57	0	80	100	32.9
		DDT	90	100	29.5
		MH + DDT	95	100	21.4
		MH	65	100	13.5
18	Apr. 6, '57	0	95	100	30.2
		DDT	95	100	28.9
		MH + DDT	90	100	14.0
		MH	90	100	20.4
20	Apr. 8, '57	0	60	100	19.8
		DDT	75	100	22.1
		MH + DDT	15	90	2.3
		MH	70	95	6.8
22	Apr. 10, '57	0	75	100	29.9
		DDT	85	95	31.2
		MH + DDT	65	100	10.5
		MH	55	80	7.5
24	Apr. 12, '57	0	65	95	29.7
		DDT	100	100	26.4
		MH + DDT	60	95	4.1
		MH	60	95	7.5
26	Apr. 14, '57	0	100	100	25.6
		DDT	100	100	28.5
		MH + DDT	70	100	2.3
		MH	90	100	9.1
28	Apr. 16, '57	0	70	100	26.8
		DDT	60	100	23.7
		MH + DDT	40	95	4.1
		MH	30	80	3.5
30	Apr. 18, '57	0	65	100	14.5
		DDT	85	100	16.2
		MH + DDT	60	95	4.2
		MH	25	95	3.4

0 = Control or no treatment.
DDT = seeds treated with dichloro-diphenyl-trichloroethane.
MH + DDT = seeds previously treated with DDT.
MH = seeds washed of DDT and then treated with maleic hydrazide.

ments did not live long. By the time the seedlings were measured for height, they were already showing definite indications that they would ultimately die. It would be interesting to find out under field conditions the relative vigor and maturity of the corn plants from seeds stored with MH for eight to 10 days.

There were faint indications starting from the 20-day storage treatment that MH affected the viability of the seeds and also the rate of emergence of the seedlings.

DISCUSSION

Fairchild (1953) has shown that at maturity, the embryo, whether that of a hybrid or an inbred, has five embryonic leaves. The death, therefore, of the treatment seedlings might be attributed to MH-interference on mitosis which prevented formation of the sixth and later foliage leaves on the stem apex normally occurring soon after onset of germination.

Seedlings grown from seeds soaked in maleic hydrazide solution grew very slowly and died sooner or later.

If ever MH is to be used as a seed treatment, the direct use of the powder form seems to be most practical. However, the results of these six experiments indicate that the possibility of using maleic hydrazide for delaying germination or delaying maturity is somewhat remote. The difference in rate of emergence of the seedlings from seeds stored with and without maleic hydrazide was little. Whenever this slight difference occurred, height and subsequent survival of the treatment seedlings were also affected. The treatment seedlings that grew slower than the control seedlings died prematurely. Seedlings grown from seeds stored with maleic hydrazide for 12 or more days also died.

The only hope now lies in hitting upon a maleic hydrazide dosage (amount of and length of storage with MH) that would actually delay flowering although no indications of any MH effect are apparent during the early seedling stage. For this purpose, the six to eight days storage in experiment 6 should serve as a good guide in planning future experiments.

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NOTE: A PRACTICAL METHOD OF COLLECTING SOIL NEMATODES FOR POPULATION SURVEYS

A basic requirement in many nematological investigations is a technique that facilitates qualitative as well as quantitative determinations of soil nematode populations.

In cases where a large number of soil samples are to be examined, Stockli's [1] direct examination technique and Cobb's [2] and Baerman's [3] methods have been found too slow and time consuming. The inactivity and sluggishness of some nematodes in soil and their mortality in the bottom of the funnel, due to the time required for extraction, are drawbacks to the Baerman funnel technique and Christie and Perry's [4] modification of it. Cobb's method was found to be inadequate for clay and loam soil types. A modification of these techniques, which gains some of their advantages and eliminates some of their drawbacks, has been found satisfactory for some Philippine soils.

The writer's modified technique employs the fundamental principle of Cobb's sieving along with Baerman's funnel technique. Only one sieve which will trap adults of the known nematode parasites of field crops and vegetables may be used. The procedure is as follows: The soil samples are first weighed and mixed thoroughly by rolling them on thick paper. A 50-gram aliquot sample is weighed and soaked in water in a beaker to loosen the soil particles. The resulting soil suspension is then poured into a 200-mesh sieve. This step is repeated twice to be sure that all the nematodes are caught by the sieve. The nematodes and associated debris and remaining soil particles are washed out of the screen and again sieved to remove the excess materials. The nematodes and remaining debris are then washed into a 250-milliliter beaker. The beaker is covered with fine muslin cloth or ordinary cheese cloth and placed upside-down into a funnel filled with water (fig. 1). Practically all of the nematodes will pass through the muslin into the funnel in four

[1] A. Stockli, *Ber. Schw. Bot. Ges.*, **54** A, 160 (1943).
[2] N. A. Cobb, U.S. Dept. Agric. Bur. Pl. Ind., Agric. Tech. Cir. 1 (1918).
[3] G. Baerman, *Med. Geneesk. Lab. Welteverden. Feestbundel.* (1917). *Cited in Natura Jutlandica* **1**: 271-278. 1948.
[4] J. R. Christie and V. G. Perry, *Proc. Helminth. Soc. Wash.*, **18**: 106-108 (1951).

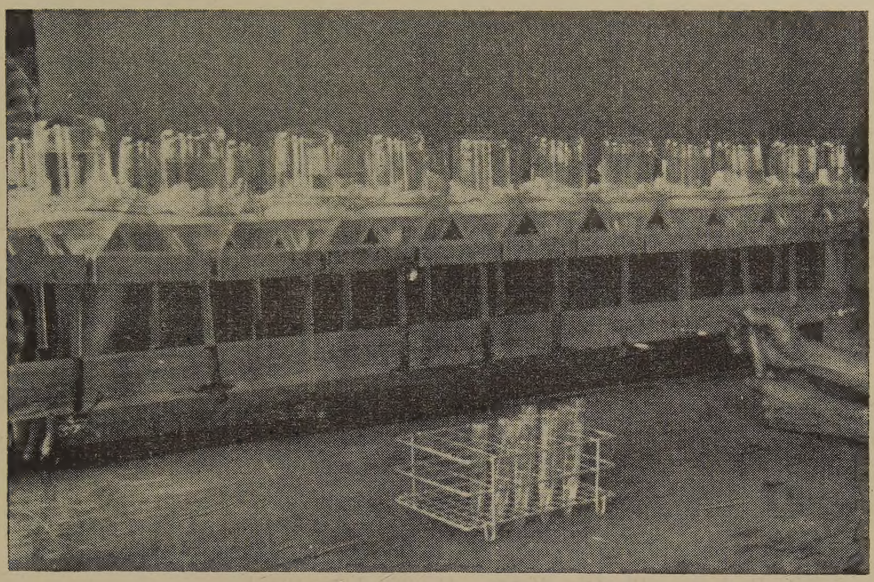

Fig. 1.—A set-up for nematode extraction.

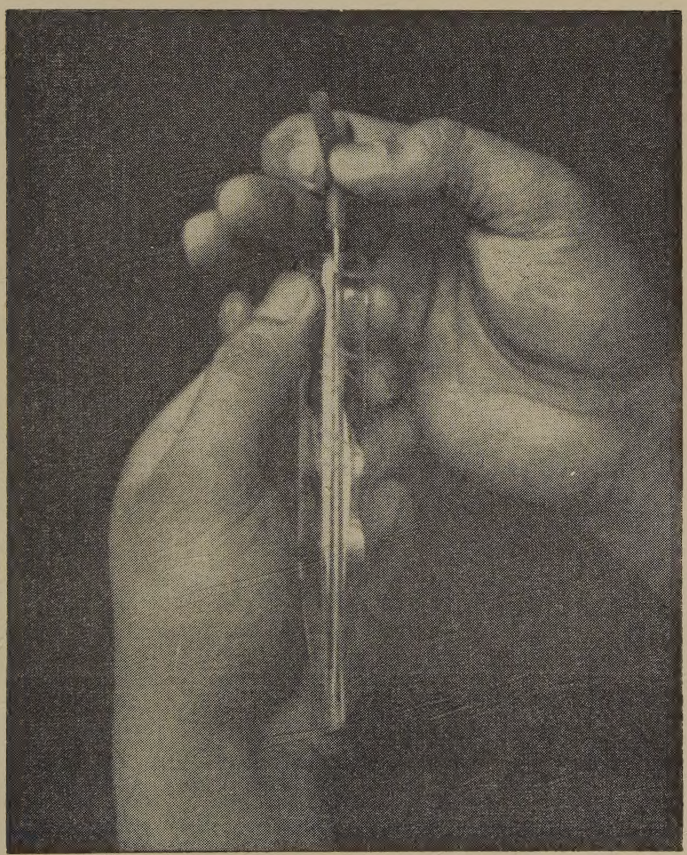

Fig. 2.—Nematodes pipetted out of a centrifuge where they have concentrated at the bottom.

to eight hours and will accumulate at the base of the stem. After the nematodes have passed through and settled at the bottom, preferably the next day, the funnel is drained into a centrifuge tube (Esser, 1957)[5] and allowed to stand for at least an hour. The accumulated nematodes are pipetted out of the tube (fig. 2) and transferred to small (United States Department of Agriculture, Bureau of Plant Industry type) syracuse watch glasses, in which they are counted and examined directly under a binocular microscope. The usual procedure for survey work is to make three extractions and counts per sample and average the results.

This method eliminates the possibility of large scale nematode escape. Moreover, it minimizes the interference from organic debris frequently encountered in Cobb's decanting method. When done on a routine basis, the method is also reasonably fast.

—Cesar P. Madamba

[5] R. P. Esser, Pl. Dis, Reptr., 41, 269-270 (1957).

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